How to use SEC-SAXS (UPLC (Waters ACQUITY)　2018.11.19)

1 Exchanging buffer / Equilibration of SEC column

1A Using PF's SEC column (The SEC column was already connected to UPLC system).
1B Using your SEC column.

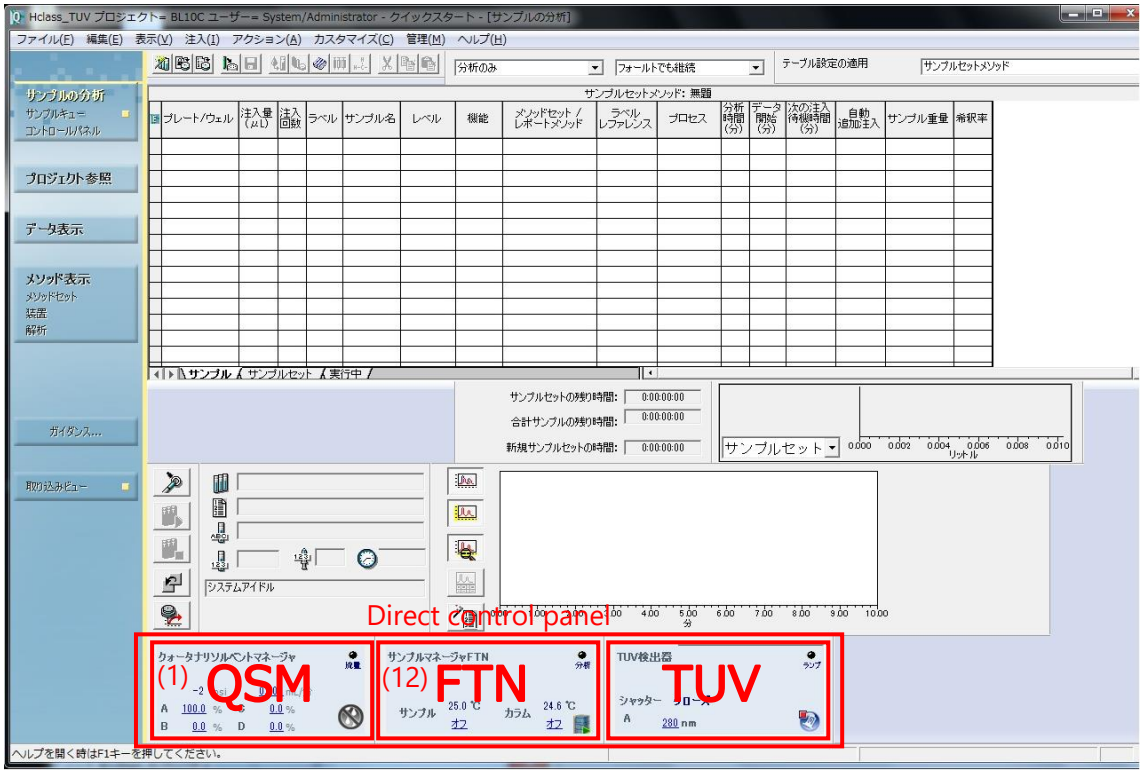
1A Using PF's SEC column (The SEC column was already connected to UPLC system).

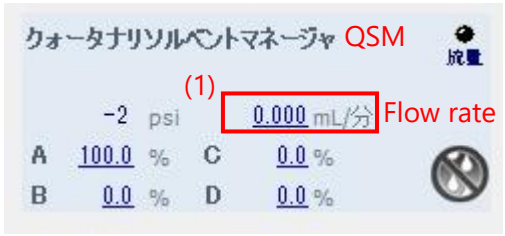
At the beginning of your BT, the BL staff set up the system as described below.

- UPLC system, UV spectrophotometer and PILATUS have been connected and started.
- The SEC column has been connected to UPLC system and equilibrated with MilliQ (or other buffer). The flow rate is set to low [for example 0.05 ml/min].
  (Buffer should be filtered and degassed. Degassing your buffer @Biophysics & Molecular physiology Lab. of PF is recommended.)
- Empower3 (UPLC control software) can be operated by remote desktop on BL control PC.

1A-1　Flow rate window

Click the flow rate display [XXXX mL/分] (1) in the QSM (クォータナリーソルベントマネージャ:quaternary solvent manager) part of the direct control panel of the UPLC control software.
[流量設定] flow rate window will open.

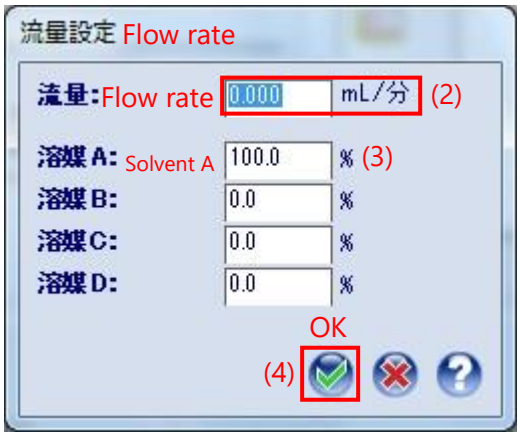




1A-2 Stopping the flow

Enter [0] mL/分 in [流量]: flow rate (2) and [100.0] % in [溶媒 A]: Solvent A (3).

Click [green check mark] (4) to stop the flow and wait several minutes until the reduction of the pressure.



1A-3 Placing the buffer bottle

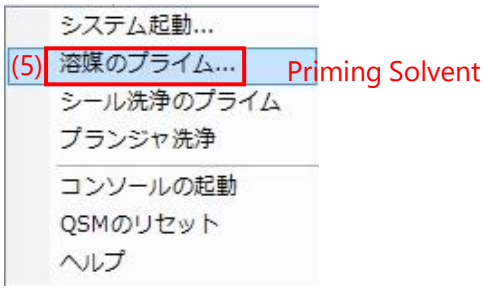
Place your buffer bottle on the tray of the UPLC system. Put [A] line (yellow labelled) into your buffer bottle, and cover the spout of the bottle with parafilm.

*Keep Purge line (orange labelled) in MilliQ bottle. [Operation rule was changed on 2018.8.27.]

1A-4 Priming Solvent window

Right-click the QSM (クォータナリーソルベントマネージャ:quaternary solvent manager) part of the direct control panel of the UPLC control software and select [溶媒のプライム]: Priming Solvent (second line) (5) .

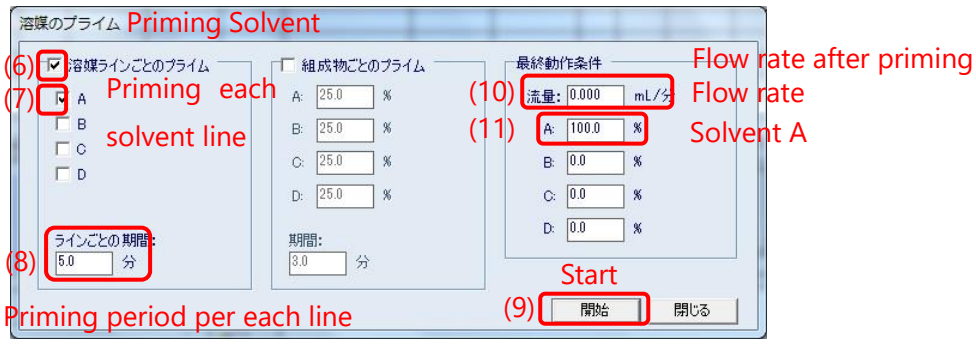
[溶媒のプライム]: Priming Solvent window will open.



1A-5 Priming Solvent [A] line

In [溶媒のプライム]: Priming Solvent window, check [溶媒ラインごとのプライム]: Priming each solvent line (first line in the left side column) (6), check [A] line (second line in the left side column) (7). and enter [5] minute in [ラインごとの期間]: Priming period (bottom line on the left side column) (8).

Click [開始]: Start (9), [A] line solvent will exchange. (Proceed 1A-7 without the finish of this step.)



1A-6 Equilibration of the SEC column

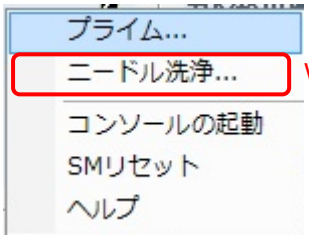
After the priming [A] line solvent, set the flow rate as described 1A-1 and 1A-2, and start the

equilibration of the SEC column.（for example: Superdex 200 increase 10/300: 0.4 ~0.5 ml/min）Equilibrate the column completely.

*If you enter the flow rate (10) and the composition of solvent (11) at the final condition area: right side column, the UPLC will continue working. When you want to stop the UPLC after priming, you should enter [0] ml/min at the final condition area: right side column.

1A-7 Washing needle

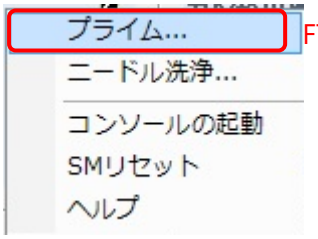
Right-click the FTN (Sample Manager with Flow-Through Needle) part (12) of the direct control panel of the UPLC control software and select [ニードル洗浄] : washing needle (second line) (13). Enter [6] second for washing period, and click [OK]. Washing needle in sample manager will start.

(13) 

Washing needle

1A-8 FTN priming window

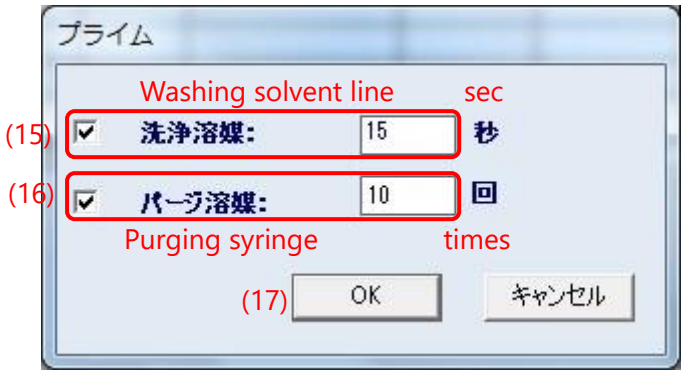
Right-click the FTN (Sample Manager with Flow-Through Needle) part (12) of the direct control panel of the UPLC control software and select [プライム] : FTN priming (first line) (14).

(14) 

FTN priming

1A-9 FTN priming

In the priming window, enter [15] sec in [洗浄溶媒]: washing solvent line (15) and [10] times in [パージ溶媒] in purging syringe (16) and click [OK] (17). Priming injector will start.（Proceed 1A-10 and after without the finish of 1A-9.)



1A-10　Sample temperature setting

Right-click [オフ] (blue letters) (18) next to サンプル：sample temperature in the FTN part of the direct control panel of the UPLC control software. [値設定]: temperature window will open, enter sample temp value (4 ~ 60℃)(19) and click [green check mark] (20).

* If you enter [0], the sample temperature is set to ambient temperature.

1A-11　Column temperature setting

Right-click [オフ] (blue letters) (21) next to カラム：column temperature in the FTN part of the direct control panel of the UPLC control software. [値設定]: temperature window will open, enter column temp value (4 ~ 60℃) (19) and click [green check mark] (20).

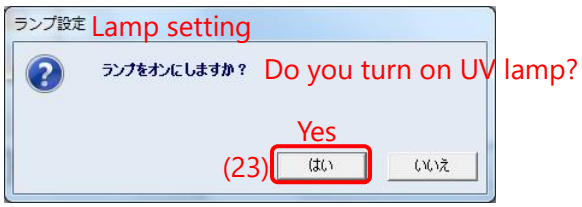
* If you enter [0], the column temperature is set to ambient temperature.

1A-12　Checking UV lamp status

Check the indicator on the upper-right corner of the TUV part of the direct control panel of the UPLC control software (Green: UV lamp ON, Black: UV lamp OFF).

1A-13　Turning on UV lamp

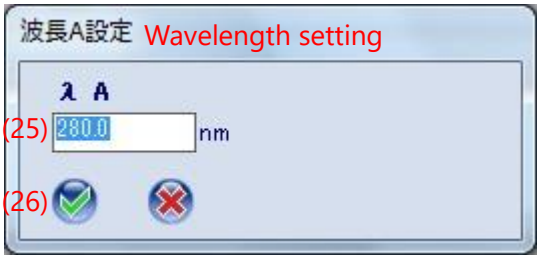
If the UV lamp is OFF, click the lamp icon on the lower-right corner (22). [ランプ設定]: Lamp setting window will open. Click [はい]:Yes (23), and UV lamp will turn on.



1A-14　Changing wavelength of UV detector

If you need to change wavelength of UPLC UV detector. Right-click the wavelength number on the TUV part (24) of the direct control panel of the UPLC control software. [波長 A 設定]: wavelength setting window will open.

Enter the wavelength (25) and click [green check mark] (26).



※When the column is equilibrated with other buffer, perform step 1A-1~9 with MilliQ [A line] and equilibrate the column with MilliQ for 5 minute. And then perform step 1A-1~9 with new buffer [A line]. When you want to change buffer from ligand-free buffer to new ligand-plus buffer, you could perform step 1A-1~9 with new ligand-plus buffer immediately.

1B Using your SEC column.

At the beginning of your BT, the BL staff set up the system as described below.

- UPLC system, UV spectrophotometer and PILATUS have been connected and started.
- UPLC system is equilibrated with MilliQ and the flow rate is set to low [for example 0.05 ml/min].
- Empower3 (UPLC control software) can be operated by remote desktop on BL control PC.

CAUTION: The equilibration of column at Lab. is recommended to save time.

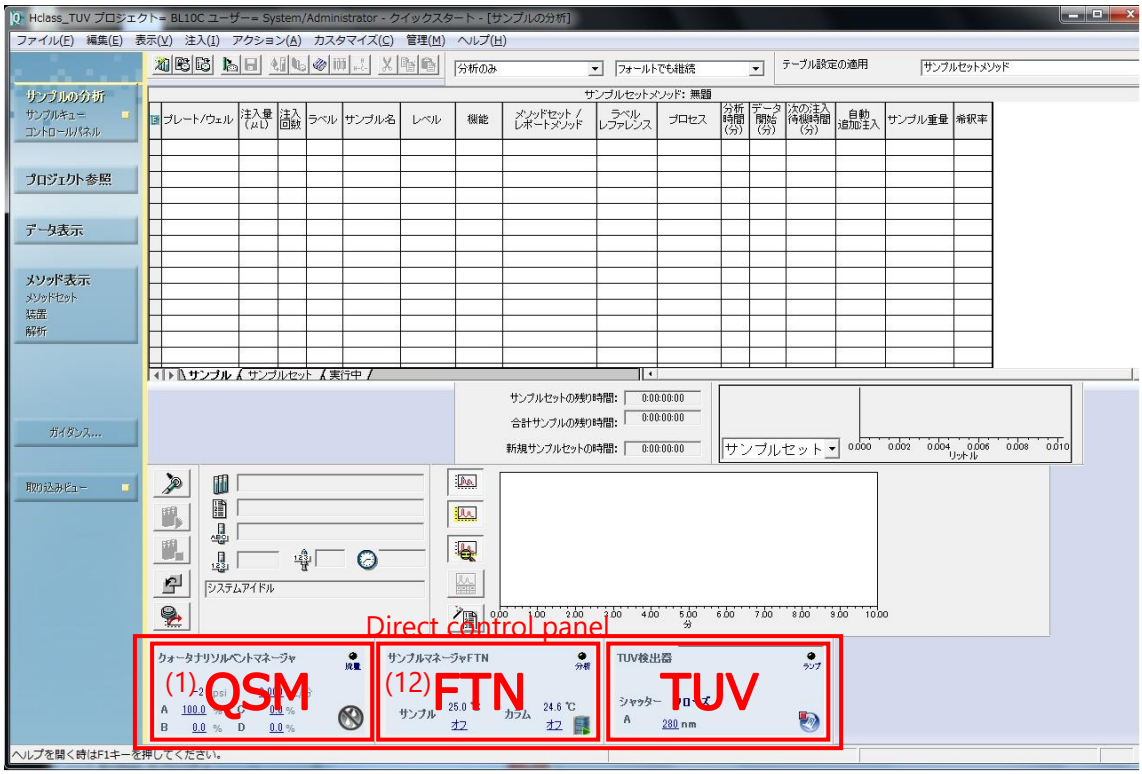
※When the column is stored with 20% ethanol etc.

Perform step 1B-1~7with MilliQ [A line] and then wash column with more than 1 column volume MilliQ to eliminate ethanol in the column. Perform step 1A-1~14 with buffer [A line] and start the equilibration of column.

WARNING: Use filtered and degassed buffer：(<0.2 um filter). To degass buffer @Biophysics & Molecular physiology Lab. of PF again is recommended.

1B-1　　Flow rate window

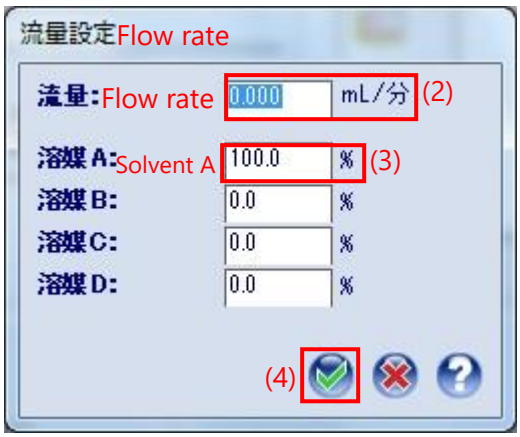
Click the flow rate display [XXXX mL/分] (1) in the QSM (クォータナリーソルベントマネージャ:quaternary solvent manager) part of the direct control panel of the UPLC control software. [流量設定]: flow rate window will open.



1B-2　　Stopping the flow

Enter [0] mL/分 in [流量]: flow rate (2) and [100.0] % in [溶媒 A]: Solvent A (3).

Click [green check mark] (4) and wait several minutes until the reduction of pressure.



1B-3 Placing the buffer bottle

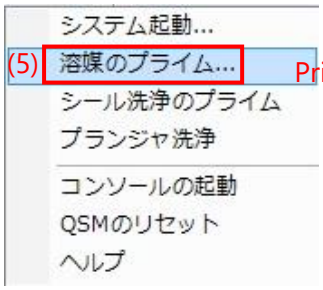
Place your buffer bottle on the tray of the UPLC system. Put [A] line (yellow labelled) into your buffer bottle, and cover the spout of the bottle with parafilm.

*Keep Purge line (orange labelled) in MilliQ bottle. [Operation rule was changed on 2018.8.27.]

1B-4 Priming Solvent window

Right-click the QSM (クォータナリーソルベントマネージャ:quaternary solvent manager) part of the direct control panel of the UPLC control software and select [溶媒のプライム]: Priming Solvent (second line) (5) .

[溶媒のプライム]: Priming Solvent window will open.

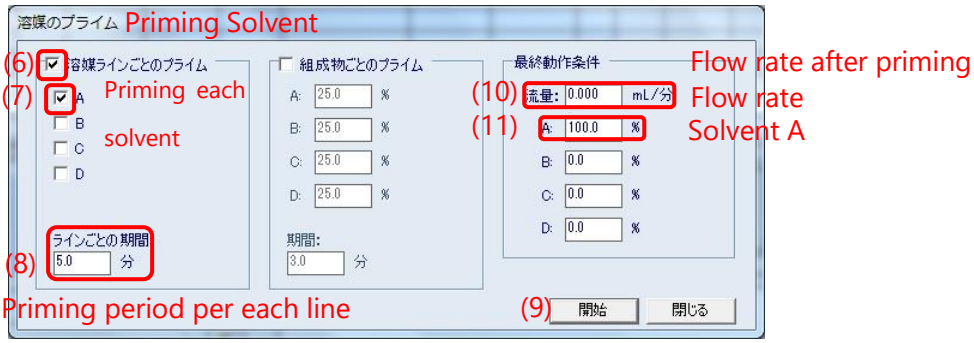


1B-5 Priming Solvent [A] line

In [溶媒のプライム]: Priming Solvent window, check [溶媒ラインごとのプライム]: Priming each solvent line (first line in the left side column) (6), check [A] line (second line in the left side column) (7). and enter [5] minute in[ラインごとの期間]: Priming period (bottom line on the left side column) (8).

Click [開始]: Start (9), [A] line solvent will exchange. (Proceed 1B-7 without the finish of this

step.)



1B-6 Equilibration of the SEC column

After the priming [A] line solvent, set the flow rate as described 1B-1 and 1B-2, and start the equilibration of the UPLC system to remove the remaining buffer.（for example: 0.4 ~0.5 ml/min for 5minutes）

*If you enter the flow rate and the composition of solvent at the final condition area: right side column, the UPLC will continue working. When you want to stop the UPLC after priming, you should enter [0] ml/min at the final condition area: right side column.

1B-7 Setting up the SEC column

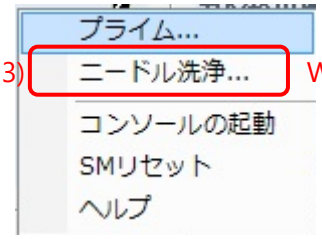
Set the flow rate to 0.2 ml/min as described 1B-1 and 1B-2. Connect your column between orange PEEK tube (upstream) and black PEEK tube (downstream).

(The connector is 1/16 inches connector（No.10-32UNF, Tricorn column (GE) could connect directly.）

Set the appropriate flow rate and start the equilibration of the column.

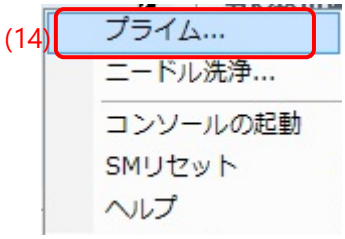
1B-8 Washing needle

Right-click the FTN (Sample Manager with Flow-Through Needle) part (12) of the direct control panel of the UPLC control software and select [ニードル洗浄] : washing needle (second line) (13). Enter [6] second for washing period, and click [OK]. Washing needle in sample manager will start.



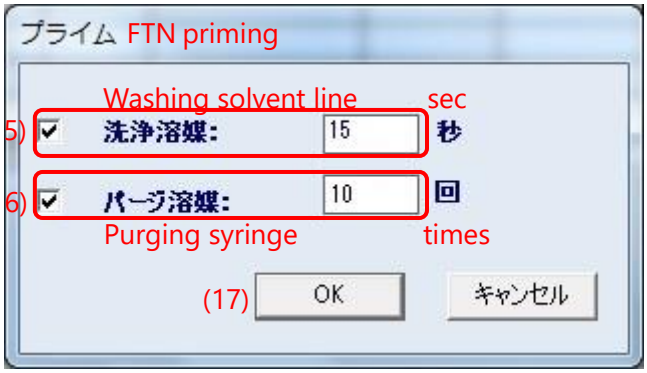
1B-9　FTN priming window

Right-click the FTN (Sample Manager with Flow-Through Needle) part of the direct control panel of the UPLC control software and select [プライム] :FTN priming (first line) (14). FTN priming window will open.



1B-10　FTN priming

In the FTN priming window, enter [15] sec in [洗浄溶媒]: washing solvent line (15) and [10] times in [パージ溶媒]: purging syringe (16) and click [OK] (17). Priming injector will start. （Proceed 1B-11 and after without the finish of 1B-10.)



1B-11　Sample temperature setting

Right-click [オフ] (blue letters) (18) next to [サンプル]: sample temperature in the FTN part of the direct control panel of the UPLC control software. [値設定]: temperature window will open, enter sample temp value (4 ~ 60℃) (19) and click [green check mark] (20).

* If you enter [0], the sample temperature is set to ambient temperature.

1B-12　Column temperature setting

Right-click [オフ] (blue letters) (21) next to [カラム]: column temperature in the FTN part of the direct control panel of the UPLC control software. [値設定]: temperature window will open, enter column temp value (4 ~ 60℃) and click [green check mark].

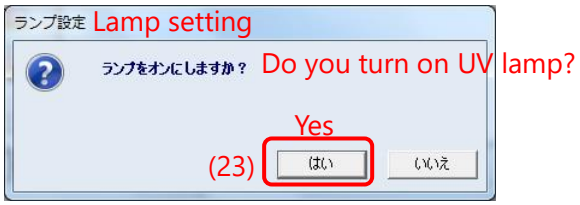
* If you enter [0], the column temperature is set to ambient temperature.

1B-13 Checking UV lamp status

Check the indicator on the upper-right corner of the TUV part of the direct control panel of the UPLC control software (Green: UV lamp ON, Black: UV lamp OFF).

1B-14 Turning on UV lamp

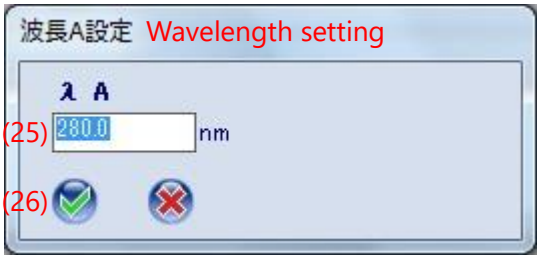
If the UV lamp is OFF, click the lamp icon on the lower-right corner (22). [ランプ設定]: Lamp setting window will open. Click [はい]:Yes (23), and UV lamp will turn on.



1B-15 Changing wavelength of UV detector

If you need to change wavelength of UPLC UV detector. Right-click the wavelength number on the TUV part (24) of the direct control panel of the UPLC control software. [波長 A 設定]: wavelength setting window will open.

Enter the wavelength (25) and click [green check mark] (26).



2 Preparation of SAXS flow cell and sample vials

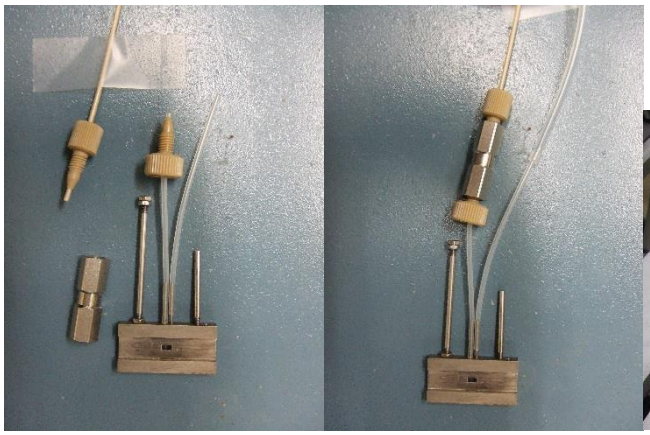
2A Preparation of SAXS flow cell.

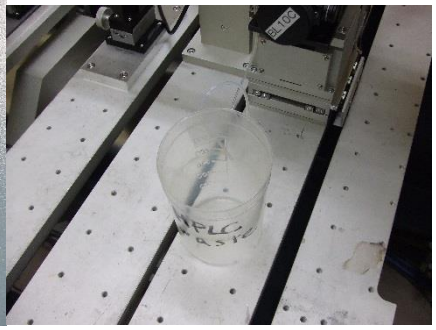
2A-1

If you want to perform the absolute scattering intensity calibration with water, you need to perform measurements of dark, air, empty cell and MilliQ, and then wash and dry SAXS flow cell with peristaltic pump and air pump.

2A-2 Connecting SAXS flow cell and tubing

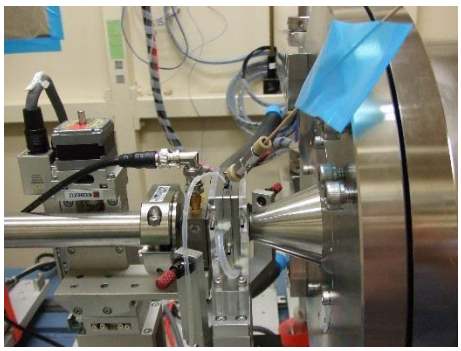
Connect the inlet of SAXS flow cell to PEEK tube (cream color) of UPLC with the fittings and union, and connect the outlet of SAXS flow cell to silicone tube. And set the waste cup on experimental bench. Fix the waste cup and silicone tube with adhesive tape.

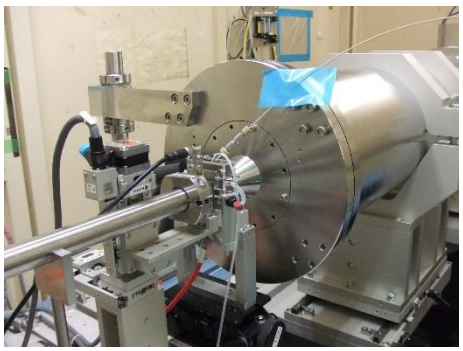




2A-3 Setting up SAXS flow cell and tubing

Check no air bubbles in the flow cell window and flow path. And set flow cell in the cell holder properly. To avoid tilting the cell position, fix the PEEK tube with the adhesive tape.





2B Preparation of the sample vial

2B-1 Dispensing sample solution into sample vial

Samples should be filtered or supernatant after centrifugation (15krpm 15min).

Dispense sample (the minimum volume is the injection volume + 10 ul) into the exclusive glass vial (water maximum recovery (P/N: 186000385c)).

(The maximum injection volume is 250 ul for UPLC.)

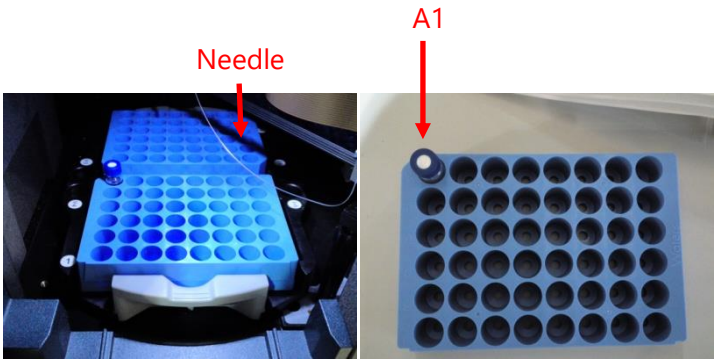
Avoid making air bubbles in the bottom narrow section. (The yellow tip is useful for dispensing sample.)

2B-2 Setting up sample plate

Open the front door of FTN, and the plate 1 will move this side. Take the plate 1 out by pulling the grey handle.

The A1 position is the cutout side of the plate 1. Set the sample vial to the plate 1 and return the plate (A1 should be the left back side), and push the handle and close the door again.

Before taking the plate, pull the grey handle to avoid touching the injecting needle (wire).



SEC-SAXS measurement

To measure SEC-SAXS data, three equipment (UPLC, UV spectrophotometer, PILATUS) are needed. Three options are offered to start them.

3 Manual mode

Each three equipment are started manually. (After the start of UPLC, click run icons of PILATUS and UV spectrophotometer.)

4 PILATUS-UV spectrophotometer synchronization mode

After the start of UPLC, click run icon of PILATUS. UV spectrophotometer will start automatically.

5 Synchronization mode

After the start of UPLC, PILATUS and UV spectrophotometer will start automatically.

(2017.06.08 Manual mode is default setting.)

PILATUS is controlled from BL measurement PC. UPLC and UV spectrophotometer are controlled with the remote desktop outside experimental hatch [UPLC: BL control PC, UV spectrophotometer: BL measurement PC.]

3 Manual mode

Each three equipment are started manually. (After the start of UPLC, click run icons of PILUTUS and UV spectrophotometer.)

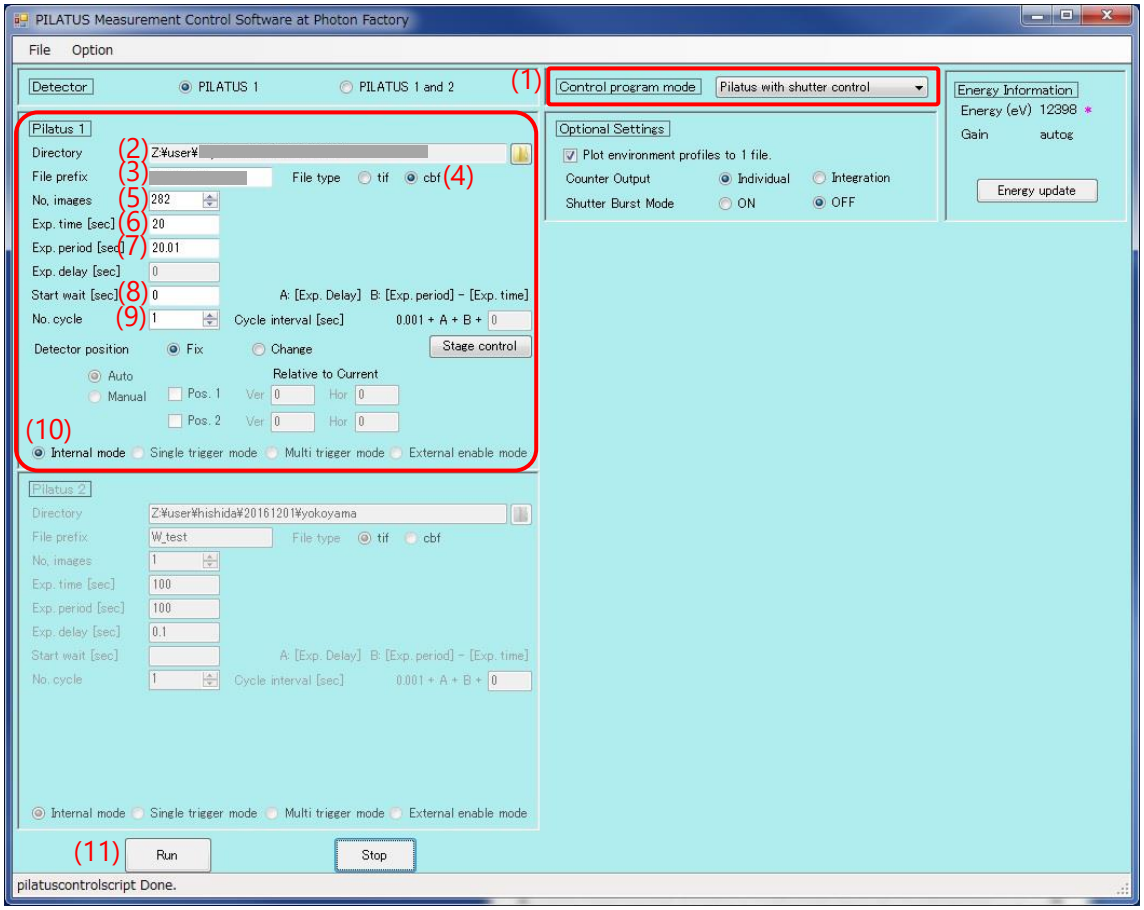
3A SAXS measurement of the background (buffer)

3A-1 Lowering the flow rate

After the equilibration of the column, lower the flow rate to 0.05 ml/min ((same as measure at peak fraction.) 0.1 ml/min@BL-15A2) and wait several minute until the pressure will be stable.

3A-2 Confirmation [Control program mode] of PILATUS Measurement Control Software

Set [PILATUS with shutter control] in [Control program mode] (1) of PILATUS Measurement Control Software.



3A-3 Measurement of background images

Measure SAXS image of background (buffer) [for example 20sec x 15 times@BL-10C, 10sec x 15 times@BL-15A2].

Set parameters in [PILATUS Measurement Control Software] as described below.

Directory [enter your folder] (2)

File Prefix [enter your file prefix] (3)

File type [select cbf or tif] (4) (If you want to process the data by other than SAngler, select tif.)

No.images [15] (5)

Exp. Time [20] @BL-10C default, [[10] @BL-15A2] (6)

Exp.period [20.01] @BL-10C default, [[10.01] @BL-15A2] (7)

Exp. Delay: (This parameter is unable to be input with [PILATUS with shutter control] mode.)

Start wait [0] (For SEC-SAXS experiment) (8)

No.cycle [1] (For SEC-SAXS experiment) (9)

Check [internal mode] (10).

Close the experimental hatch to open X-ray shutter before start.

Click [Run] (11) to start measurement.

3B Setting of UV spectrophotometer software.

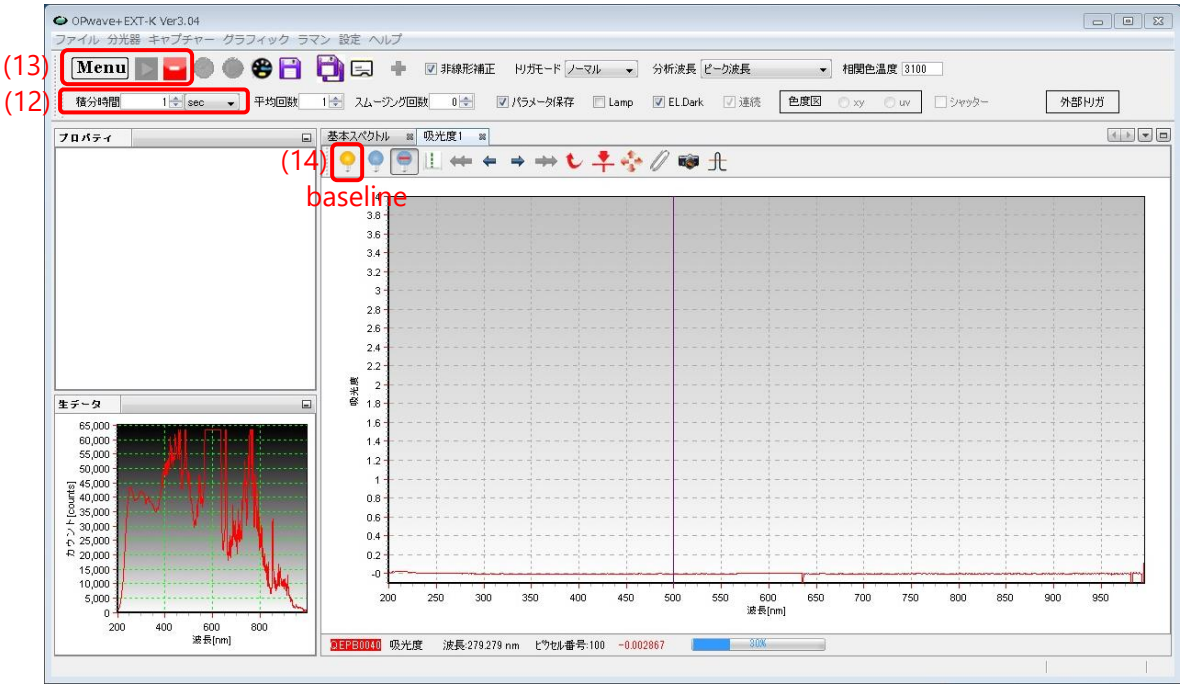
3B-1 Initial setting of UV spectrophotometer software

Confirm that [積分時間]: integration period is [1sec] (12) in OPwave+EXT-K (UV spectrophotometer software). If different period is set, the re-adjustment of the UV spectrophotometer is needed, see p.5 of OPwave manual or Appendix 4.

Confirm that OPwave+EXT-K is [live mode].

If the icon next to menu icon is grey and the spectra is renewed every sec, OPwave+EXT-K is set to [live mode].

If the icon next to menu icon is green, Click that green icon (13), and set OPwave+EXT-K to [live mode].



3B-2 measurement of reference spectra with UV spectrophotometer

Check SAXS flow cell position properly and no air bubble in the window and the flow path.

And check the flow rate is [0.05 ml/min]@BL-10C. [[0.1 ml/min] @Bl-15A2]

And then click the yellow bulb icon (14) to get reference spectra.

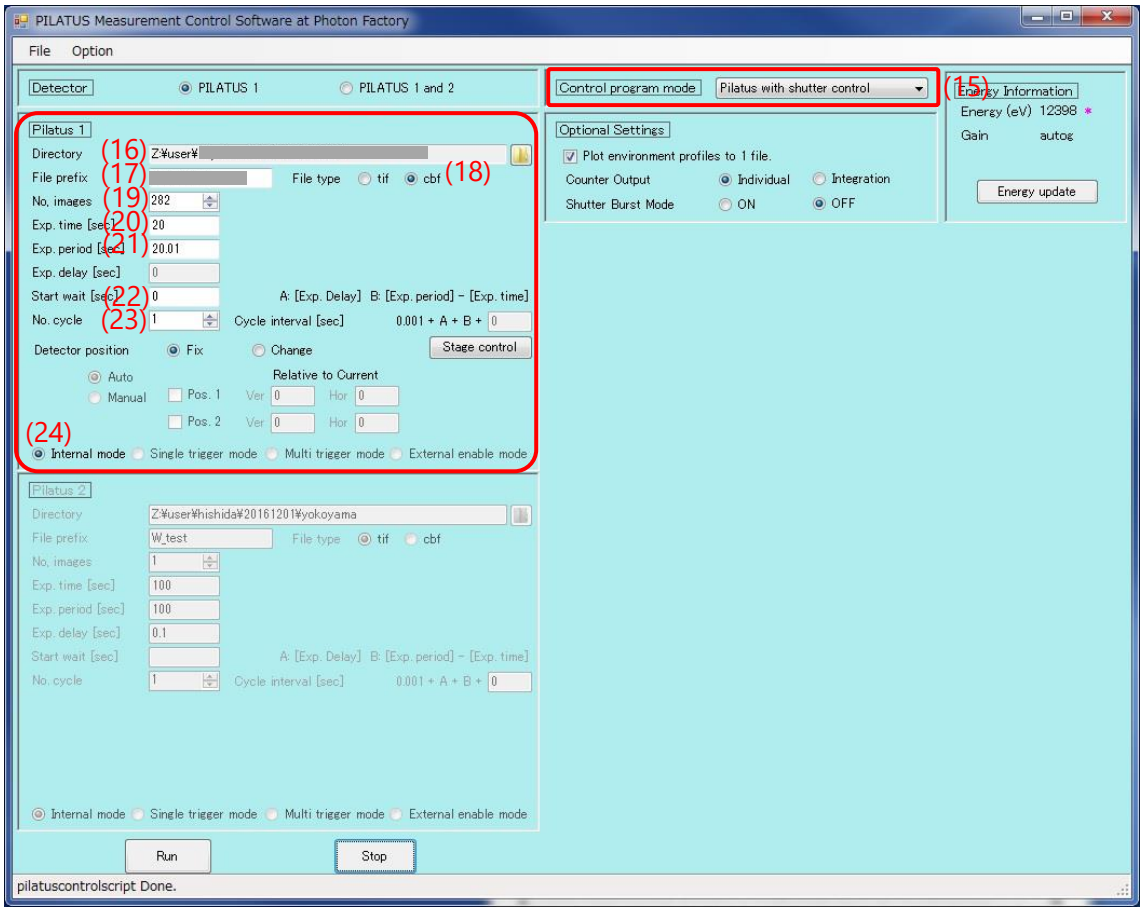
Measurement Example

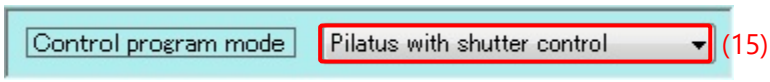
Column : Superdex200 increased 10/300 (CV: 24ml)
Flow rate: 0.5 ml/min @0~14 ml(0~28 min) and 0.05 ml/min @14~16.5 ml(28~78min) (0.1 ml/min @14~16.5 ml(28~53min) @BL-15A2).
(Measure peak fractions with slower flow rate.)

SAXS (expose 20sec/image, exp period 20.01sec)、
UV measurement period is set to 10sec as default (twice frequency as X-ray).
(@BL-10C default value. @BL-15A2 SAXS expose 10sec/image, period 10.01sec, UV 5sec)
Total number of measurements are
78 x 60 /20.01 = 234 (SAXS) [53 x 60 / 10.01 = 318 @BL-15A2]
78 x 60 /10 +1 = 469 (UV) [53 x 60 / 5 +1 = 637 @BL-15A2]

3C Setting parameters of PILATUS measurement control software

3C-1 Confirmation [Control program mode] of PILATUS Measurement Control Software
Set [PILATUS with shutter control] (15) in [Control program mode] of PILATUS Measurement Control.





3C-2　　Setting up of PILATAS measurement parameters

Set parameters in PILATUS Measurement Control Software as described below.

Directory [enter your folder] (16)

File Prefix [enter your file prefix] (17)

File type [select cbf or tif] (18) [If you want to process the data by other than SAngler, select tif.]

No.images [234] @BL-10C default, [[318] @BL-15A2] (19)

Exp. Time [20] @BL-10C default, [[10] @BL-15A2] (20)

Exp.period [20.01] @BL-10C default, [10.01 @BL-15A2] (21)

Exp. delay：　(This parameter is unable to be input with [PILATUS with shutter control] mode.)

Start wait [0] (For SEC-SAXS experiment) (22)

No.cycle [1] (For SEC-SAXS experiment) (23)

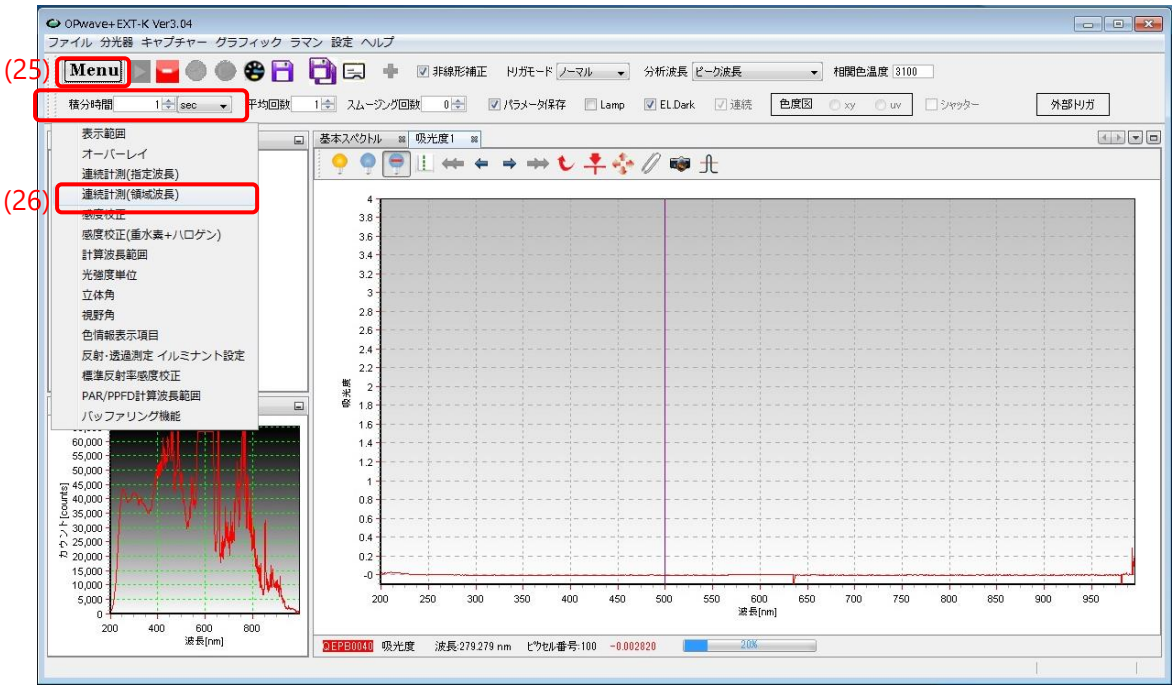
Check [internal mode] (24).

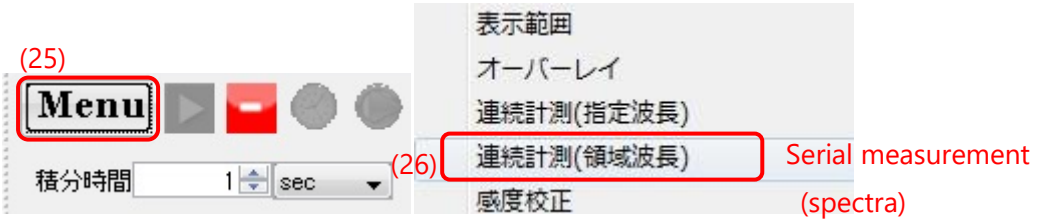
3D Setting parameters of OPwave+EXT-K

3D-1　　Setting parameters of OPwave+EXT-K

Click Menu icon (25), and select [連続計測(領域波長)]: serial measurements (spectra) (forth line) (26).

[連続計測(領域波長)]: serial measurements (spectra) window will open.





Set parameters in the window as described below.

[領域波長]: wave length range(27)　200 – 450 nm (28)

[保存ピッチ]: pitch / [全て]: all (29), Check [計測値]: measured value (30)

[計測モード]: mode / [吸光度]: absorbance (31)

[保存ファイル名]: file name / check [データ保存] :save data (32), [enter UV file name (***_UV)] (33)

[保存間隔パラメータ]: parameters for measurement period section

[保存間隔]: measurement period: [10] sec @BL-10C [[5] @BL-15A2] (34) and check [秒]:sec (35).

Check [計測回数]: No. of measurement (36), enter [469] @BL10C [[637] @BL-15A2] (37).

[ウォームアップ時間]: time for warm-up / [0] (38) and check [秒]: sec (39).

[計測開始方式]: starting method / Check [ノーマル]: Manual (40).

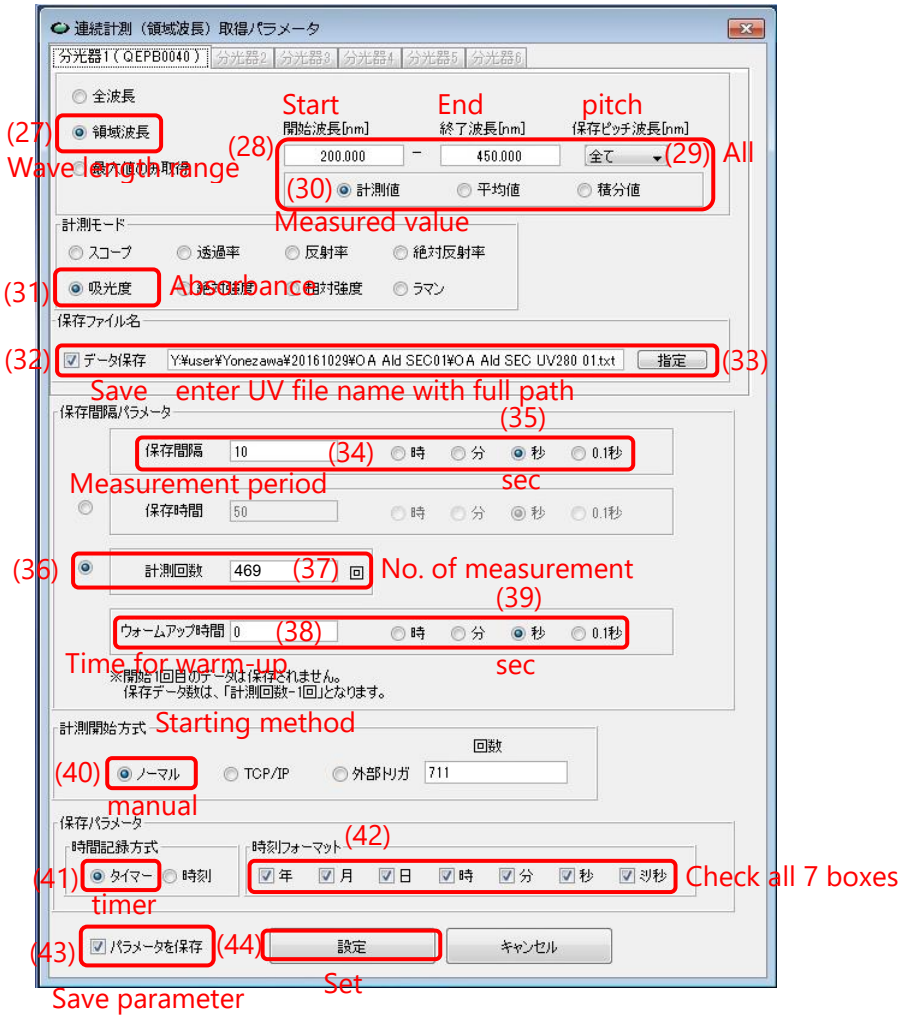
[保存パラメータ]: other parameters for saving section

Check [タイマー] : Timer(41) and Check all 7 boxes in [時刻フォーマット]: time format (42).

Check [パラメータを保存]: save parameters check box (43).

Click [設定]: Set icon (44).

Notification window will open. Click OK.



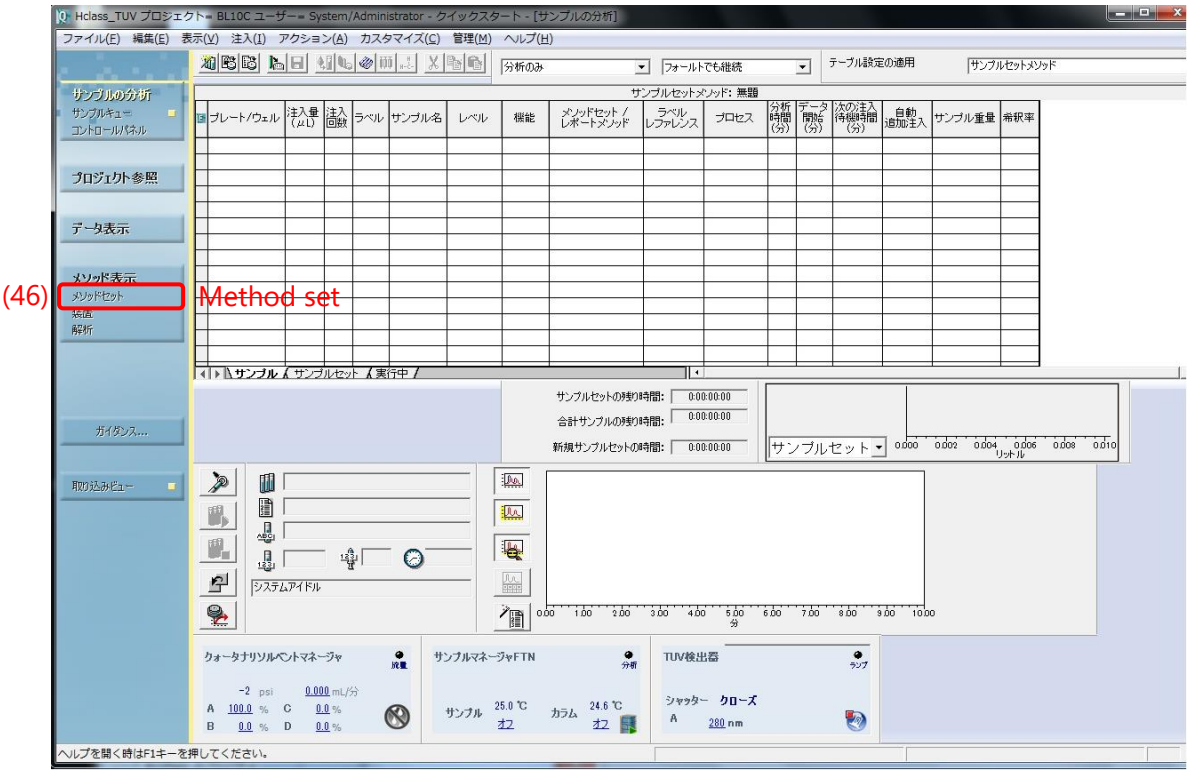
3D-2　　preparation for serial measurement

Click stop icon (red icon) (45) to stop [live mode].

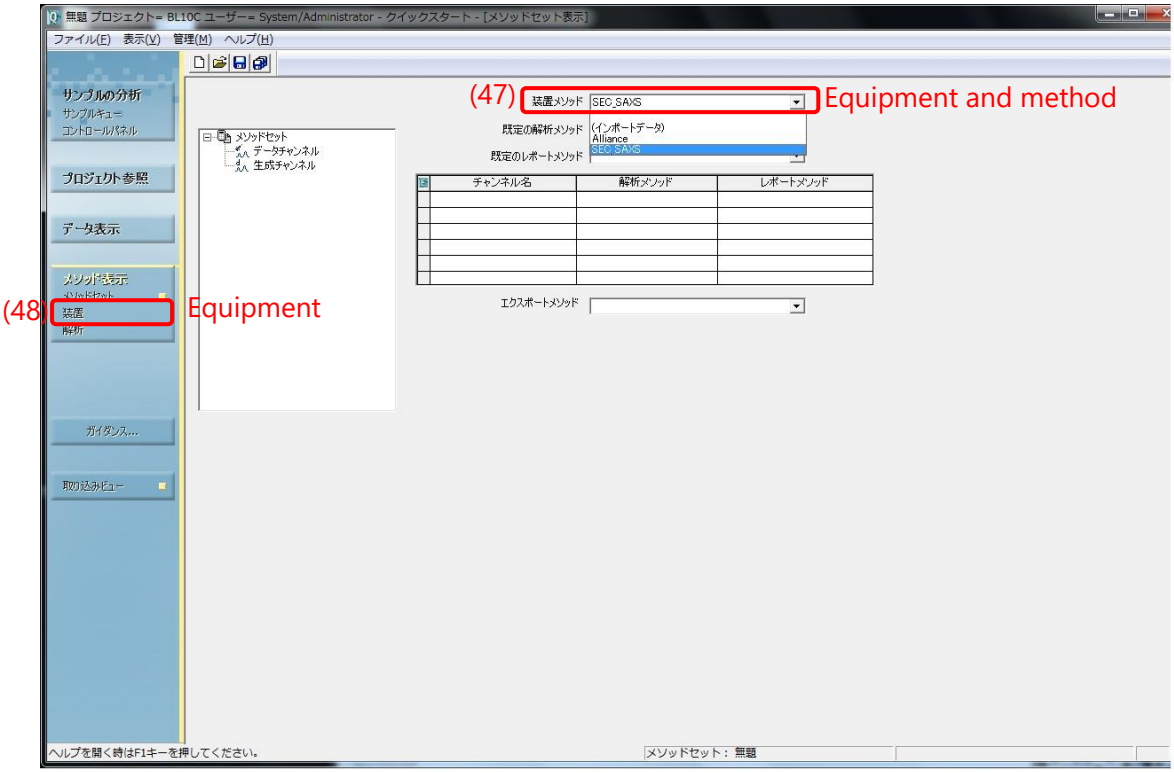
3E Setting of UPLC

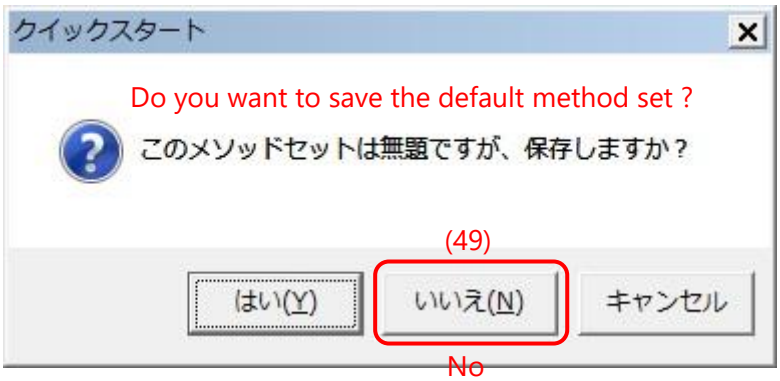
3E-1 Opening [SEC-SAXS] method

Click [メソッドセット]: Method set (46) in the navigation bar (left side of UPLC control software).



Select [SEC-SAXS] (47) in the [装置メソッド]: equipments and method, and click [装置]: equipments (48) in the navigation bar. [クイックスタート]: quick start window will open, select [いいえ]: No (49).

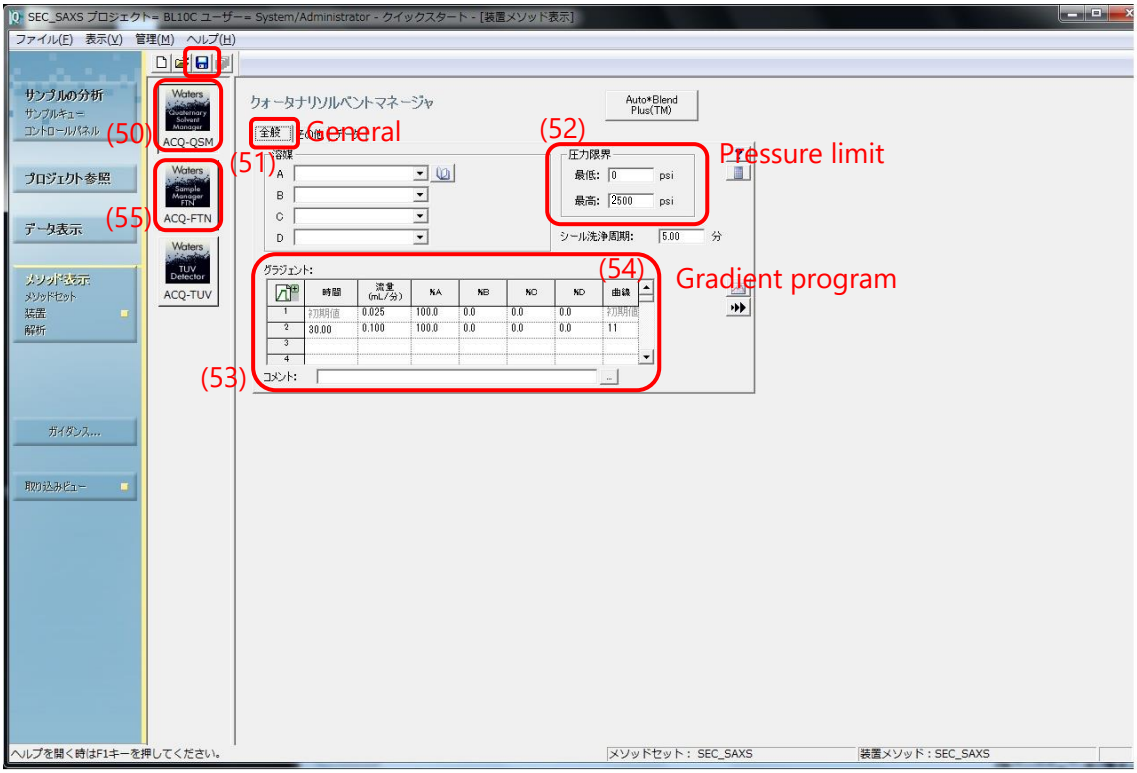


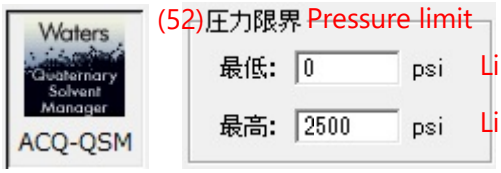


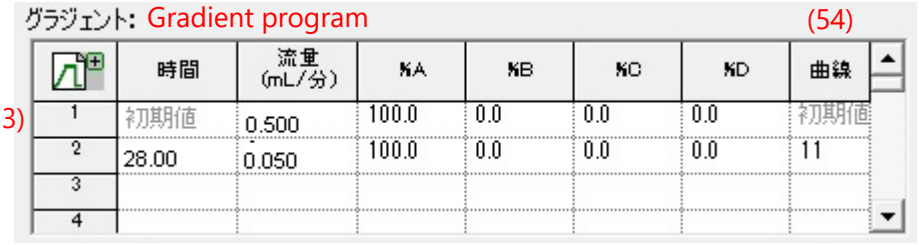
3E-2　Setting flow rate of [SEC-SAXS] method

Click [ACQ-QSM] icon (50), and move to [全般]: general tab (51).

Set [圧力限界]: pressure limits (52) (for example [最高]: max 2500 psi for Superdex200 increase 10/300)　*Pressure limit depends on the column, check manual or catalogue.







Set flowrate in the [グラジェント]: gradient section table (53).

	時間 time	流量 Flow rate	%A	%B	%C	%D	曲線 curve
1	初期値 initial	0.5	100.0	0.0	0.0	0.0	初期値 initial
2	28	0.05 0.1 [BL-15A2]	100.0	0.0	0.0	0.0	11

Set [11] in the curve field (54). ([11] means that the flow rate will change ASAP.)

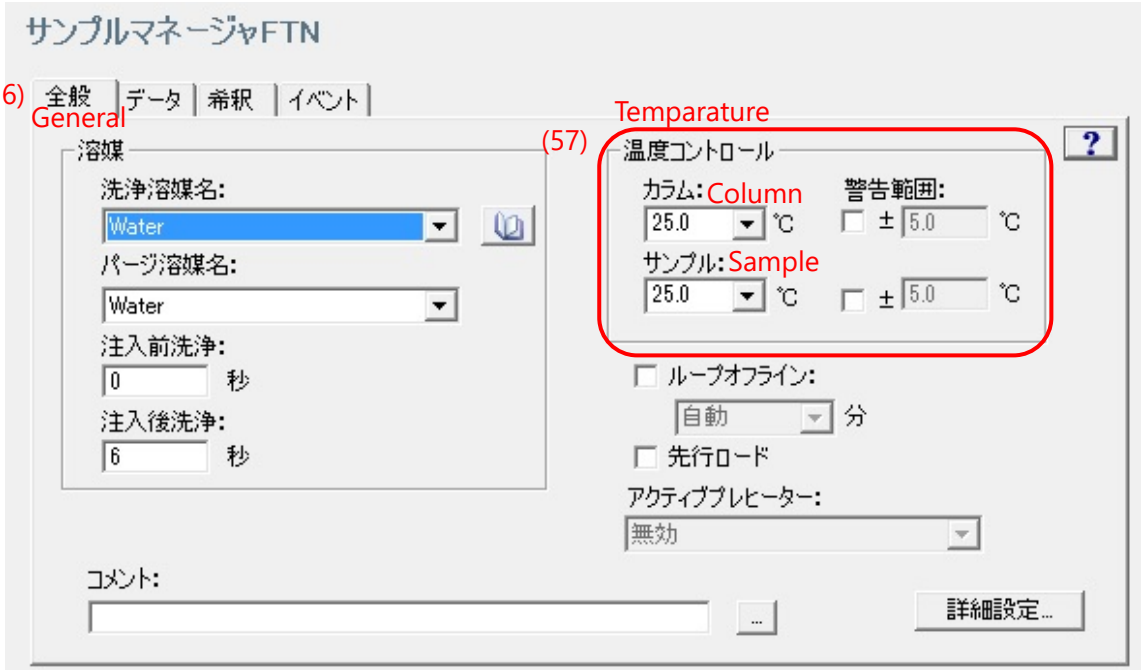
You can increase the steps by right-clicking on the table.

3E-3　　Setting temperatures of [SEC-SAXS] method

Click [ACQ-FTN] icon, and move to [全般]: general tab (56).

Set column and sample temperatures (57) in [温度コントロール]: temp section.

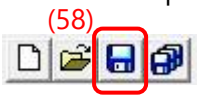
Do not check [警告範囲]: warning range section.



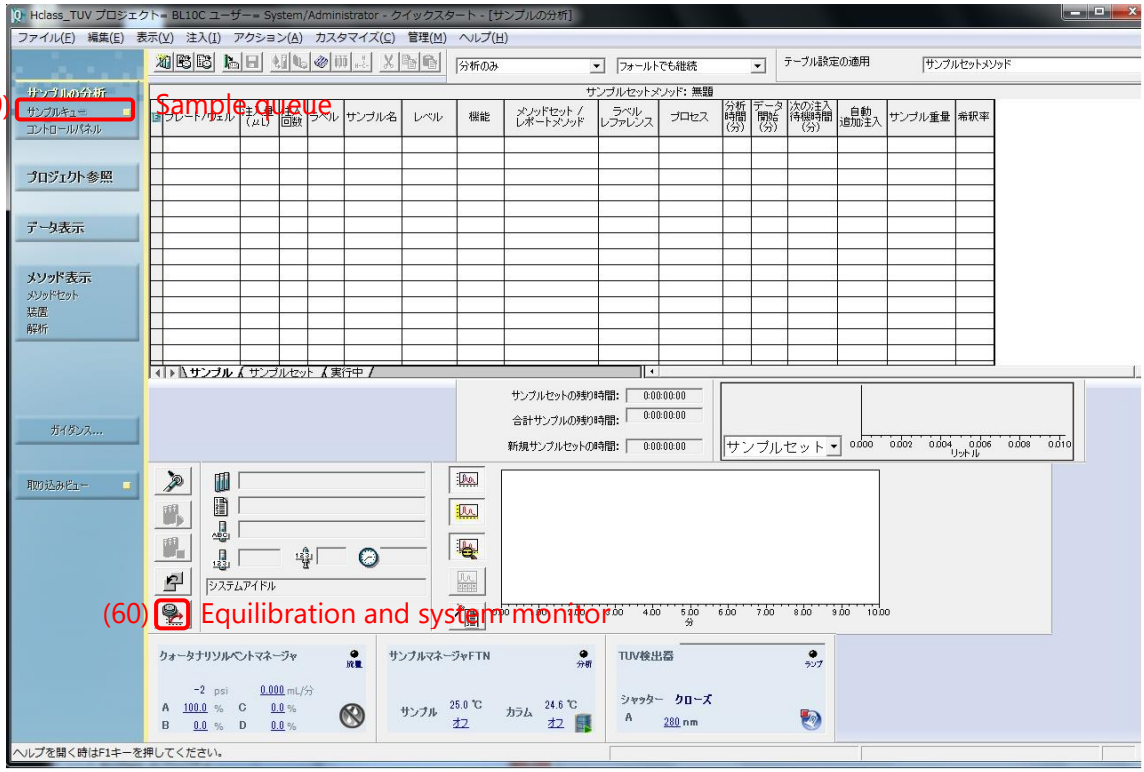
3E-4　　Saving modified [SEC-SAXS] method

Click save icon (58) to save modified method,

※To save parameters is needed to run modified method. If you do not click save icon, UPLC will not work proper method.



Click [サンプルキュー]: sample queue (59) in the navigation bar.



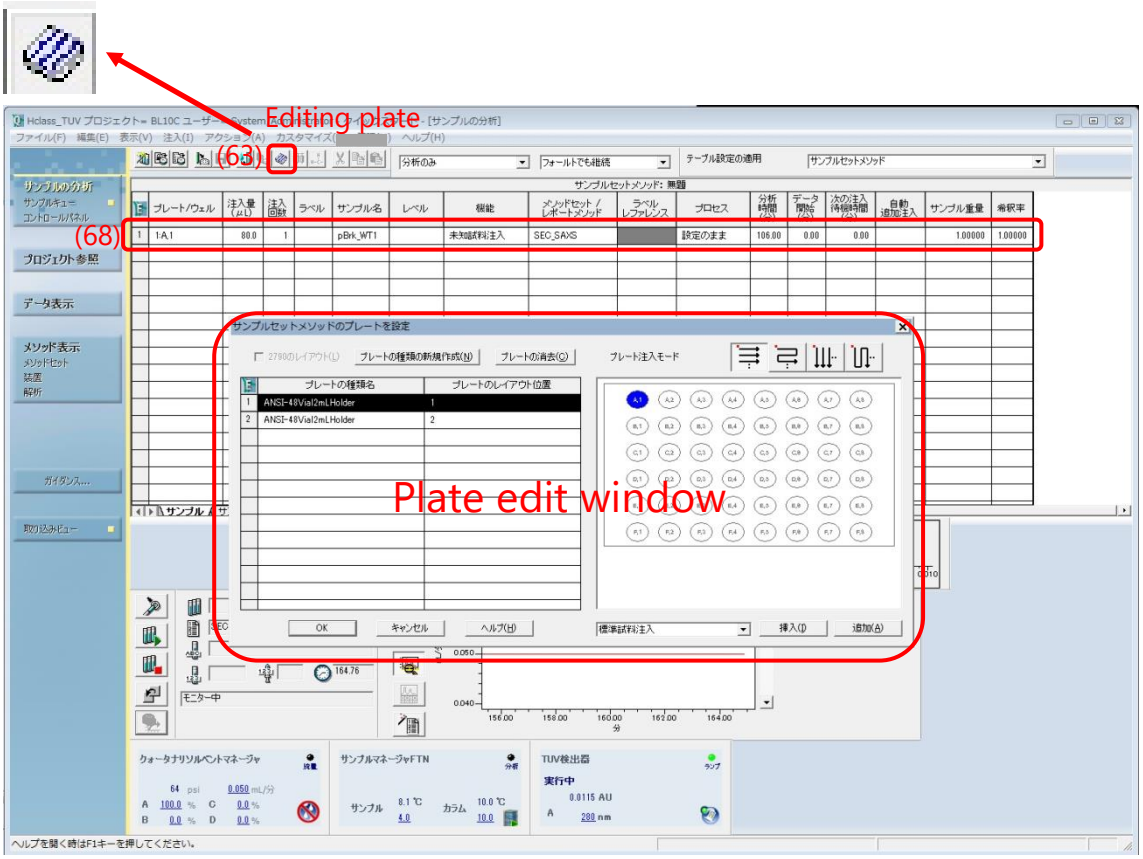
3E-5　　Starting equilibration and system monitor

Click [平衡/システムモニター]: equilibration and system monitor icon (60). [平衡/システムモニター]: equilibration and system monitor window will open.

Select [SEC_SAXS] (61) in the [装置メソッド]: equipment and method box, and click [平衡/モニター]: equilibration and system monitor button (62). Buffer will flow as the initial values of [SEC-SAXS] method (Sample injection does not start.)

3E-6 Setting up plate parameters

Click [プレート編集]: editing plate icon (63), The plate edit window will open.



In the plate edit window, select [ANSI-48 Vial 2ml Holder 1] (64) in the left side, and select [A1] position (65) in the right side.

Click [挿入]: insert icon (66) and click [OK] (67).

3E-7 Setting up injection parameters

Set parameters for the injection of UPLC (68).

Plate/well position　No. of injection　Function

Injection volume　Sample name　Method set/Report method

	プレート/ウェル (69)	注入量 (μL) (70)	注入回数 (71)	ラベル	サンプル名 (72)	レベル	機能 (73)	メソッドセット / レポートメソッド (74)
1	1:A,1	100	1		test		未知試料注入	SEC_SAXS

Unknown sample　SEC-SAXS

Analysis time

ラベル レファレンス	プロセス	分析時間 (分) (75)	データ開始 (分)	次の注入待機時間 (分)	自動追加注入	サンプル重量	希釈率
	設定のまま	78	0.00	0.00		1.00000	1.00000

[プレート/ウェル]: plate and well position / [1:A,1] (plate 1, A-1 position) (69), input have completed at 3E-6

[注入量]: injection volume (ul) / enter injection volume (max 250 ul) (70).

[注入回数]: No. of injection / [1] (71).

[サンプル名]: sample name / enter sample name (72).

[機能]: function / select [未知試料注入]: injection of unknown sample (second line) (73).

[メソッドセット/レポートメソッド]: method set and report method / select [SEC_SAXS] (74).

[分析時間]: analysis time (min) / enter [78] [[53] @BL-15A2] (75)

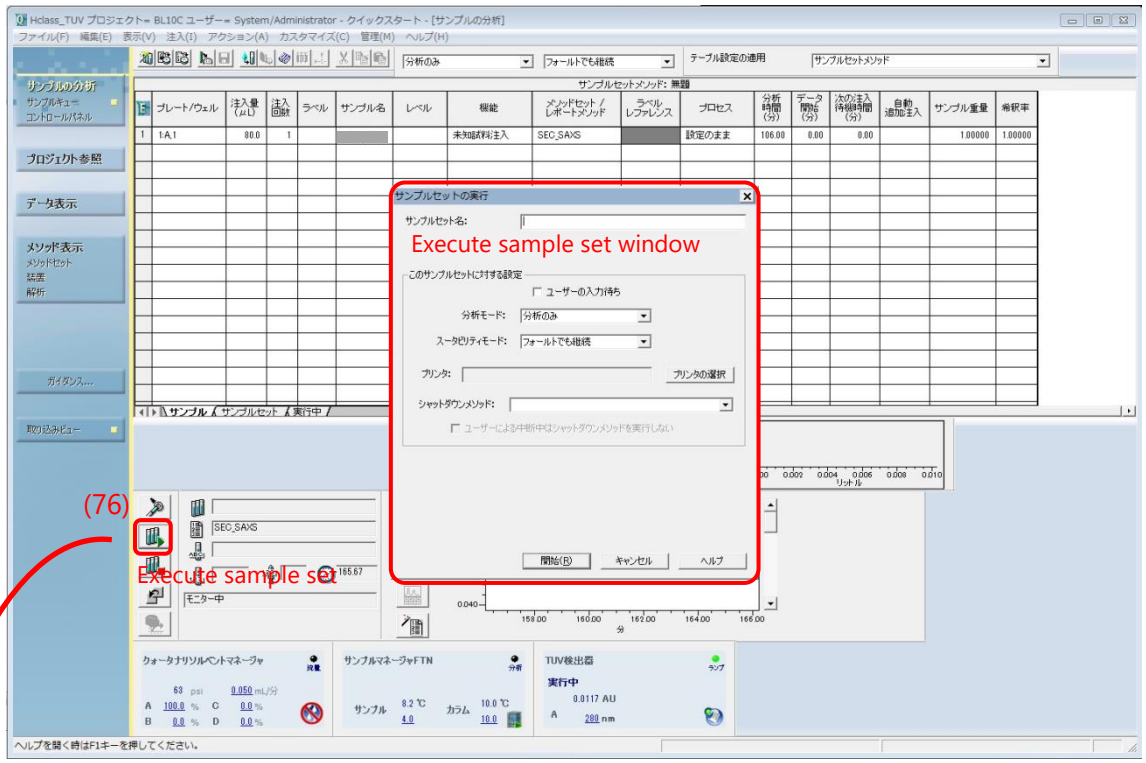
3F Execution of SEC-SAXS

3F-1 Start of the UPLC injection

Prepare SAXS flow cell and sample vial.

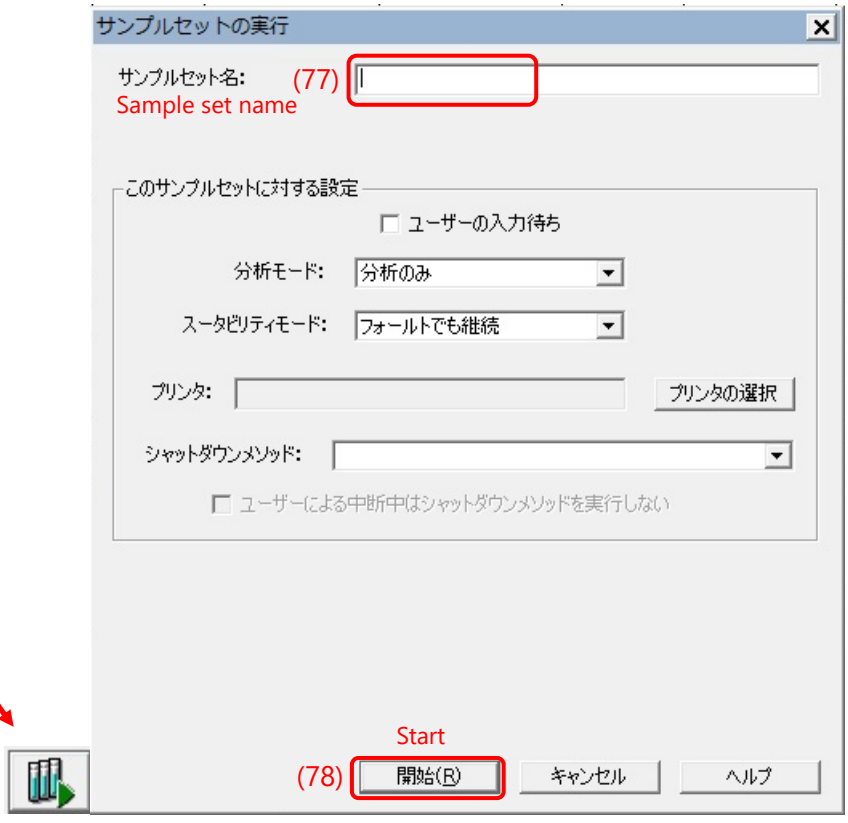
Close the experimental hatch and open DSS.

Click [execute sample set] icon (test tubes icon) (76), [execute sample set] window will open.



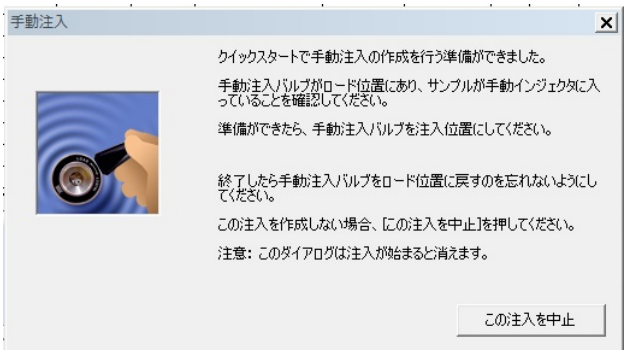
Enter [サンプルセット名]: sample set name (77) (You can set the same as the sample name and the sample set name or enter any sample set name).

Click [開始]: Start icon (78) to start injection process.



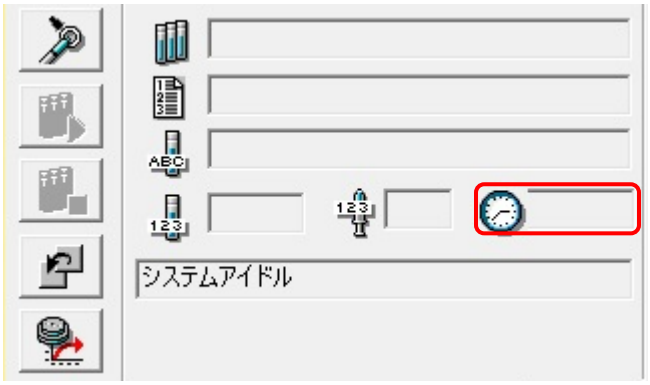
3F-2 Waiting time for the UPLC injection

The injection window will open. Just wait for the injection (waiting period:160 sec for 250 ul injection)



3F-3 Checking start of UPLC injection

Check that the injection of UPLC start (the timer starts to count up.)



Check here

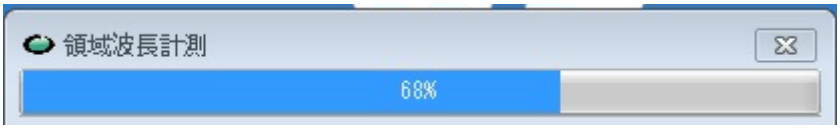
3F-4 Starting PILATUS measurement

Immediately after starting UPLC, click [Run] icon in PILATUS measurement software to start PILATUS. While PILATUS is running, a part of PILATUS measurement soft window will become pink in color and measured images will be displayed on the PILATUS mage viewer software window. (You need to change PILATUS mage viewer software to [measurement mode].)

3F-5 Starting UV spectrophotometer measurement

Immediately after starting UPLC, click [serial measurement icon] (clock icon) (79) to start UV spectrophotometer on OPwave+EXT-K. While the serial measurement of UV spectrophotometer, the progress bar window will open and update spectra on OPwave+EXT-K window.

(79)
Serial measurement

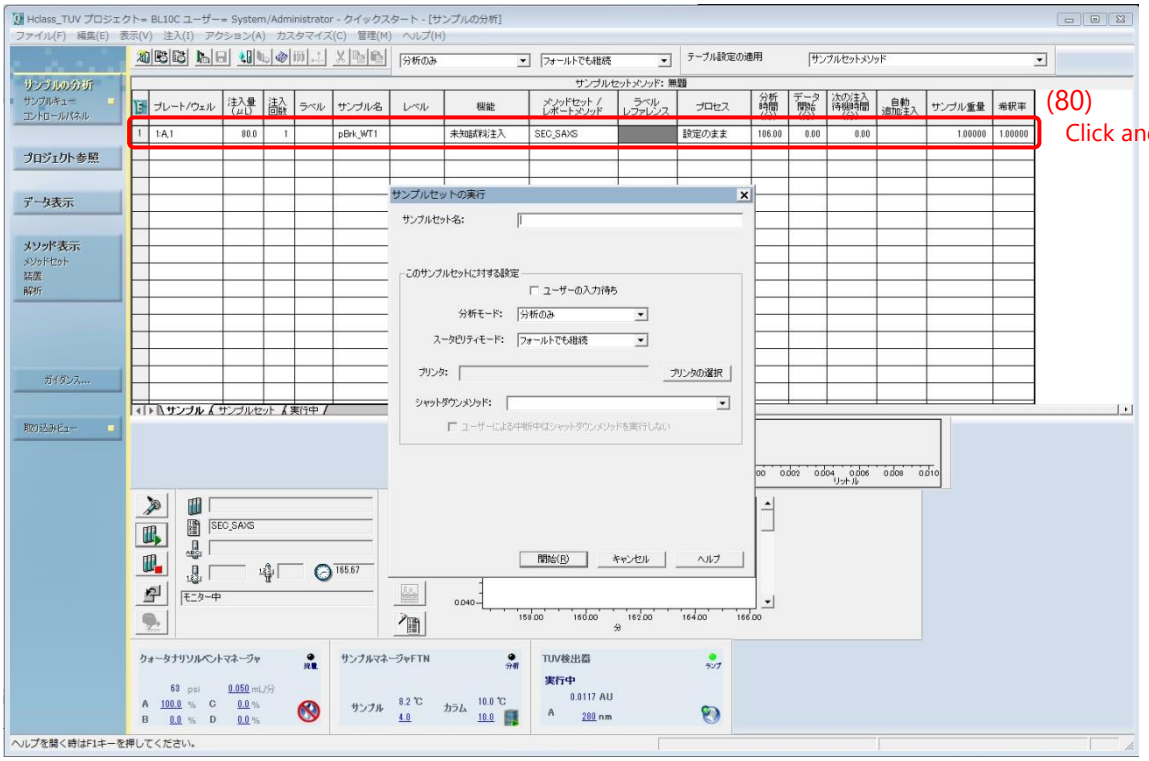


3F-6 Preparation for next measurement

After the SEC-SAXS measurement finish, increase the flow rate to re-equilibrate the column on UPLC control software (because UPLC continue the flow at final programmed condition.) Right-click the parameter line in the sample set tab and select delete (80).

CAUTION: executed sample parameters does not deleted automatically. If you forget to delete them, the same methods will be performed on next run.

And Notification window will open on OPwave+EXT-K, click OK and re-start [live mode] again by clicking green start icon (81) of OPwave+EXT-K.



(80)
Click and DEL key

(81)

Start

4 PILATUS-UV spectrophotometer synchronization mode

In this mode, you need to click run icon of PILUTUS manually after the start of UPLC injection. And then UV spectrophotometer will start automatically.

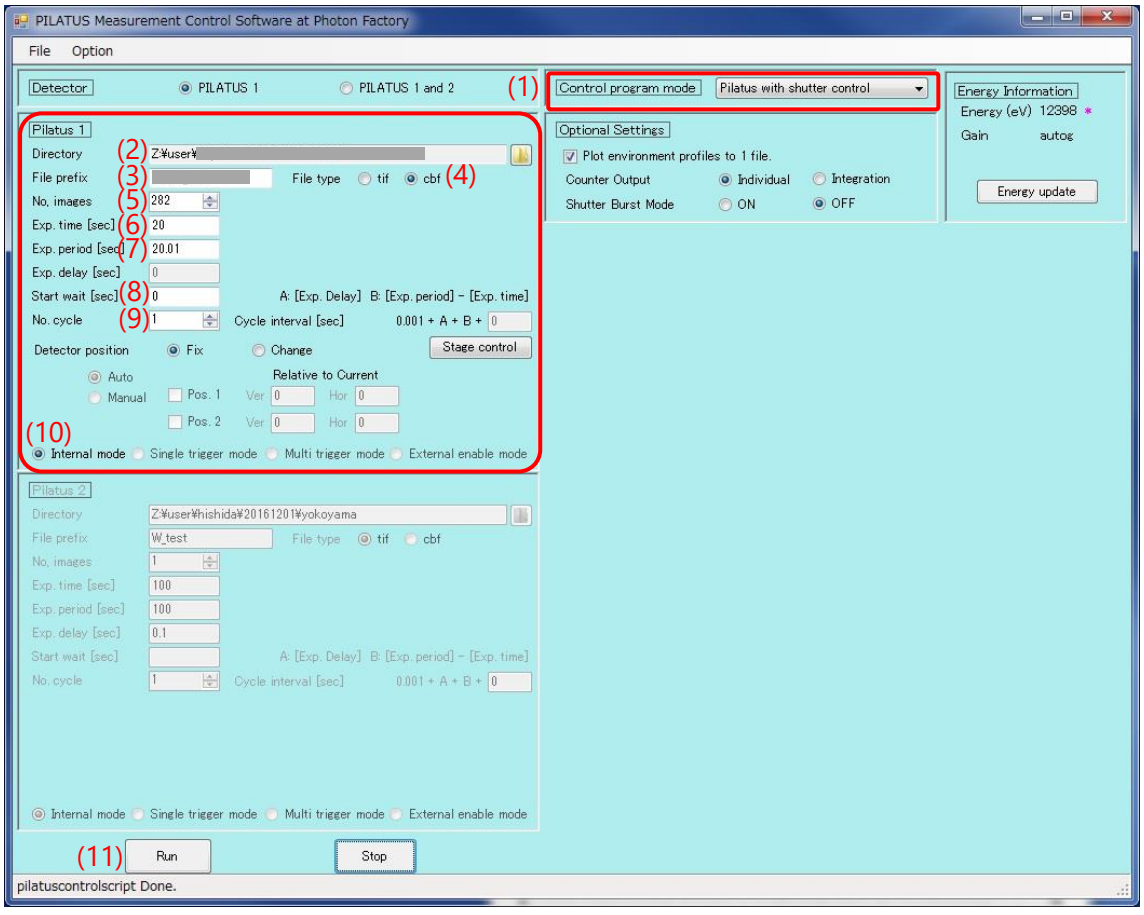
4A SAXS measurement of the background (buffer)

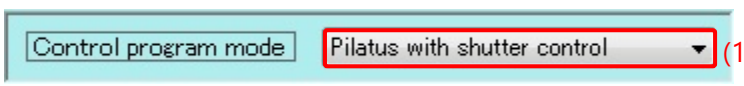
4A-1 Lowering the flow rate

After the equilibration of the column, lower the flow rate to 0.05 ml/min ((same as measure at peak fraction.) 0.1 ml/min@BL-15A2) wait several minute until the pressure will be stable.

4A-2 Confirmation [Control program mode] of PILATUS Measurement Control Software

Set [PILATUS with shutter control] in [Control program mode] (1) of PILATUS Measurement Control.





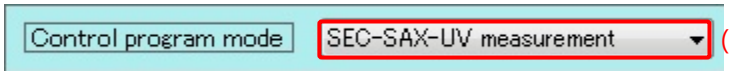
4A-3 Measurement of background images

Measure SAXS image of background (buffer) [for example 20sec x 15 times@BL-10C, 10sec x 15 times@BL-15A2].
Set parameters in PILATUS Measurement Control Software as described below.

Directory [enter your folder] (2)
File Prefix [enter your file prefix] (3)
File type [select cbf or tif] (4) (If you want to process the data by other than SAngler, select tif.)
No.images [15] (5)
Exp. Time [20] @BL-10C default, [[10] @BL-15A2] (6)
Exp.period [20.01] @BL-10C default, [[10.01] @BL-15A2] (7)
Exp. delay : (This parameter is unable to be input with [PILATUS with shutter control] mode.)
Start wait 0 (For SEC-SAXS experiment) (8)
No.cycle 1 (For SEC-SAXS experiment) (9)
Check [internal mode] (10).

Close the experimental hatch to open X-ray shutter before start.
Click [Run] (11) to start measurement.

4A-4 Changing [Control program mode]
After measurement, Set [SEC-SAXS-UV measurement] (12) in [Control program mode] of PILATUS Measurement Control to synchronize the start of PILATUS and UV spectrophotometer.



(12)

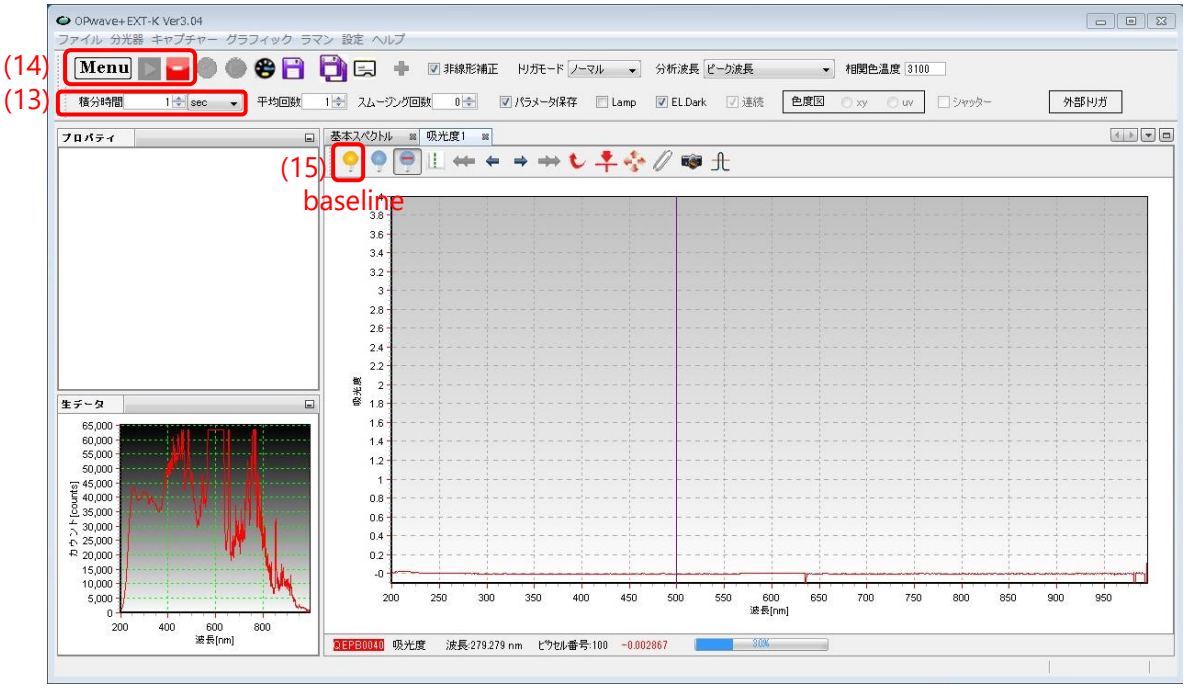
4B Setting of UV spectrophotometer software.

4B-1 Initial setting of UV spectrophotometer software
Confirm that [積分時間]: integration period is [1sec] (13) in OPwave+EXT-K (UV spectrophotometer software). If different period is set, the re-adjustment of the UV spectrophotometer is needed, see p.5 of OPwave manual or Appendix4.

Confirm that OPwave+EXT-K is [live mode].
If the icon next to menu icon is grey and the spectra is renewed every sec, OPwave+EXT-K

is set to [live mode].

If the icon next to menu icon is green, Click that green icon (14), and set OPwave+EXT-K to [live mode].



4B-2 Measurement of reference spectra with UV spectrophotometer

Check SAXS flow cell position and no air bubble in the flow path. And check the flow rate is 0.05 ml/min@BL-10C [0.1 ml/min@Bl-15A2]

And then click the yellow bulb icon (15) to get reference spectra.

Measurement Example

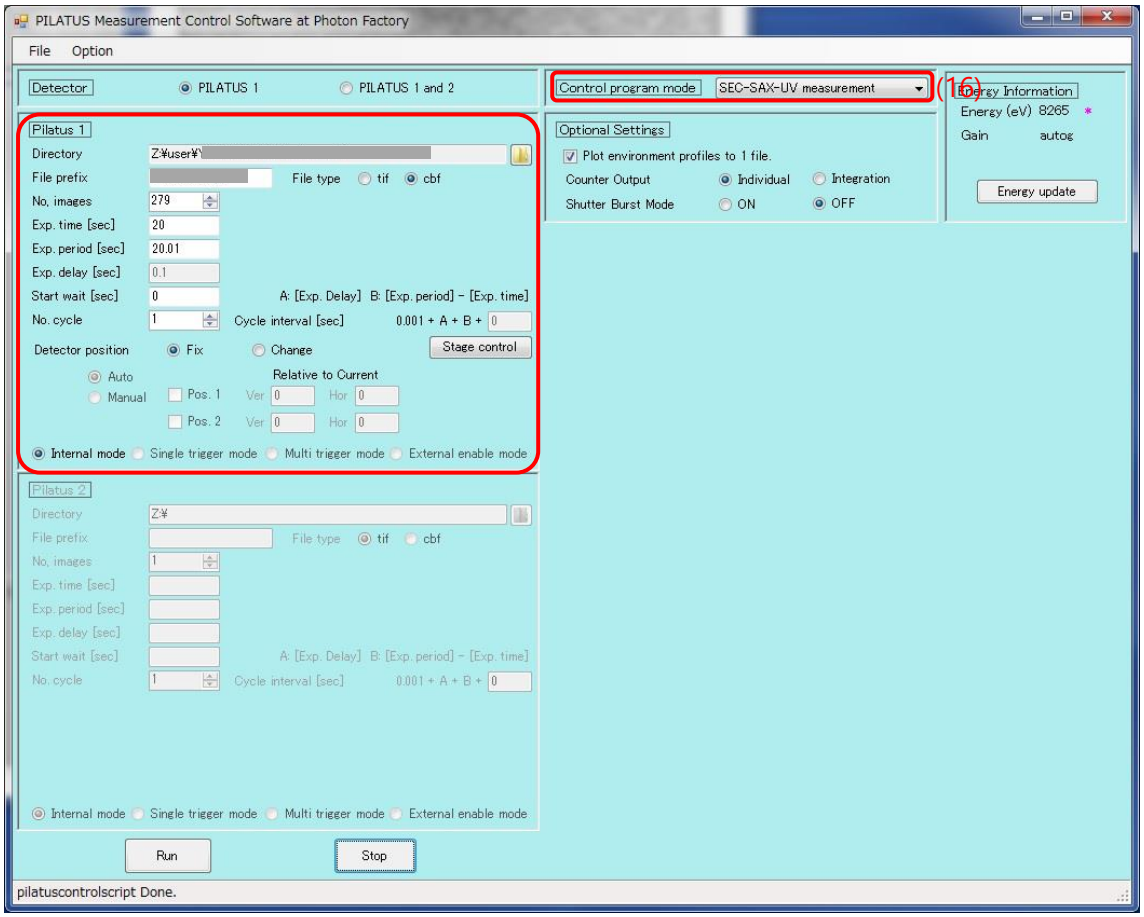
Column : Superdex200 increased 10/300 (CV: 24ml)
Flow rate 0.5 ml/min @0~14 ml(0~28 min) and 0.05 ml/min @14~16.5 ml(28~78min) (0.1 ml/min @14~16.5 ml(28~53min) @BL-15A2).
(Measure peak fractions with lower flow rate.)

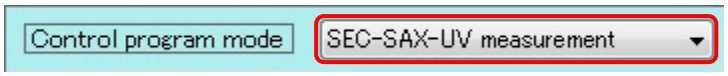
SAXS (expose 20sec/image, exp period 20.01sec)、
UV measurement period is set to 10sec as default (twice frequency as X-ray).
(@BL-10C default value. @BL-15A2 SAXS expose 10sec/image, period 10.01sec, UV 5sec)
Total number of measurements are
78 x 60 /20.01 = 234 (SAXS) [53 x 60 / 10.01 = 318 @BL-15A2]
78 x 60 /10 +1 = 469 (UV) [53 x 60 / 5 +1 = 637 @BL-15A2]

4C Setting parameters of PILATUS measurement control software

4C-1 Confirmation [Control program mode] of PILATUS Measurement Control Software

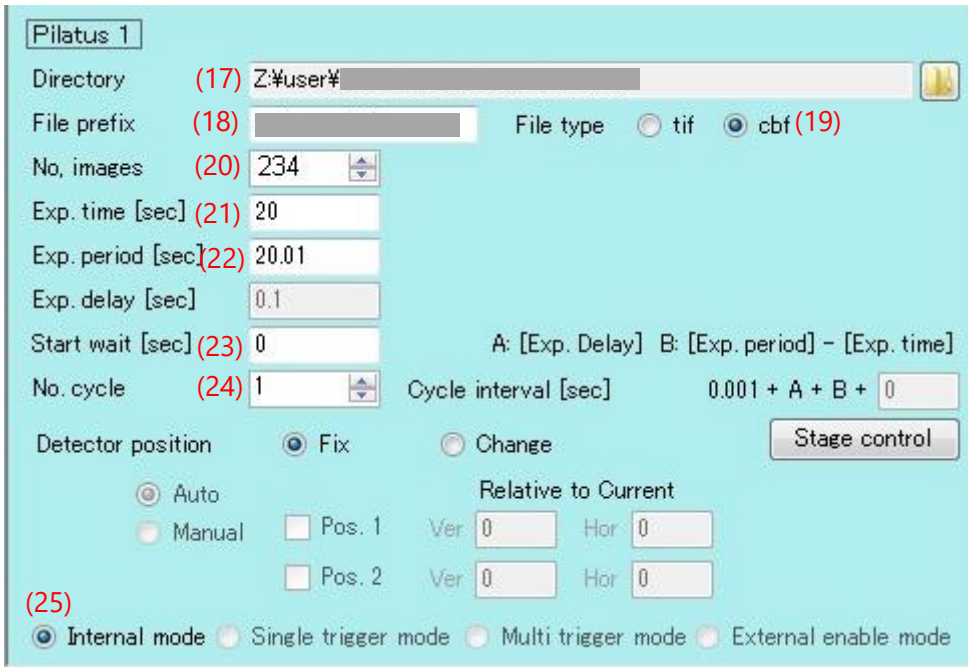
Set [SEC-SAXS-UV measurement] (16) in [Control program mode] of PILATUS Measurement Control.





4C-2　Setting up of PILATAS measurement parameters

Set parameters in [PILATUS Measurement Control Software] as described below.



Directory [enter your folder] (17)

File Prefix [enter your file prefix] (18)

File type [select cbf or tif] (19) (If you want to process the data by other than SAngler, select tif.)

No.images [234] @BL-10C [[318] @BL-15A2] (20)

Exp. Time [20] @BL-10C default, [[10] @BL-15A2] (21)

Exp.period [20.01] @BL-10C default, [[10.01] @BL-15A2] (22)

Exp. delay：(This parameter is unable to be input with [SEC-SAXS-UV measurement] mode.)

Start wait 0 (For SEC-SAXS experiment) (23)

No.cycle 1 (For SEC-SAXS experiment) (24)

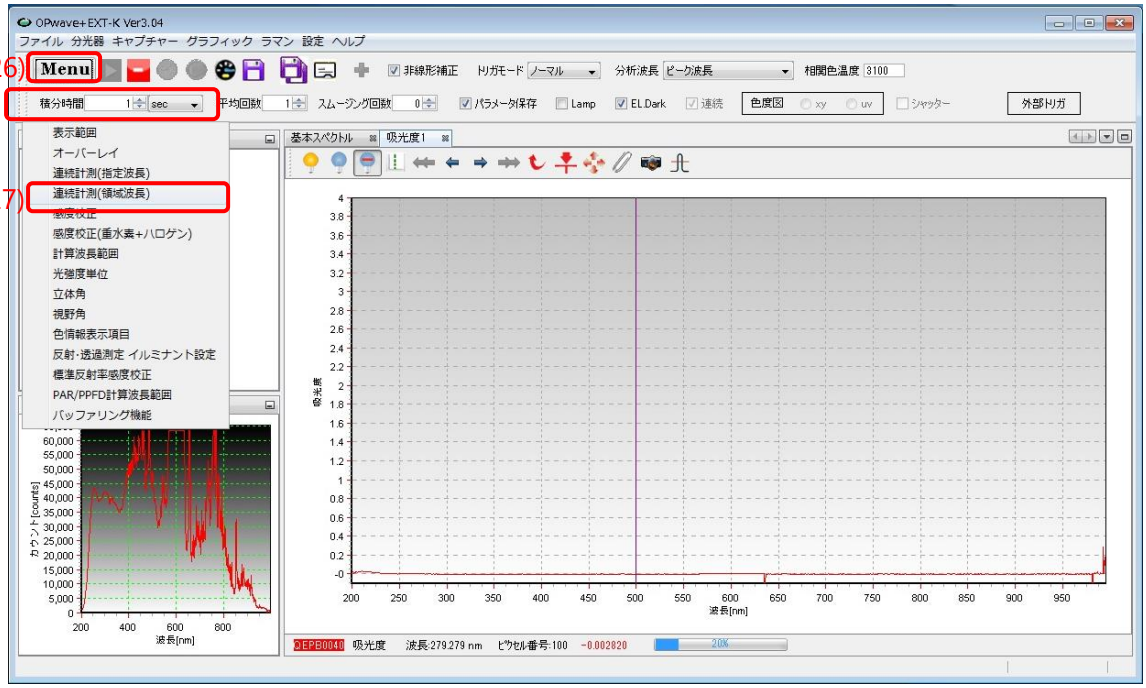
Check [internal mode] (25).

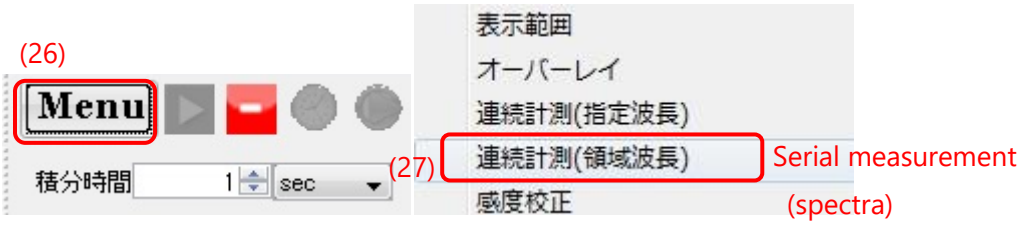
4D Setting parameters of OPwave+EXT-K

4D-1　Setting parameters of OPwave+EXT-K

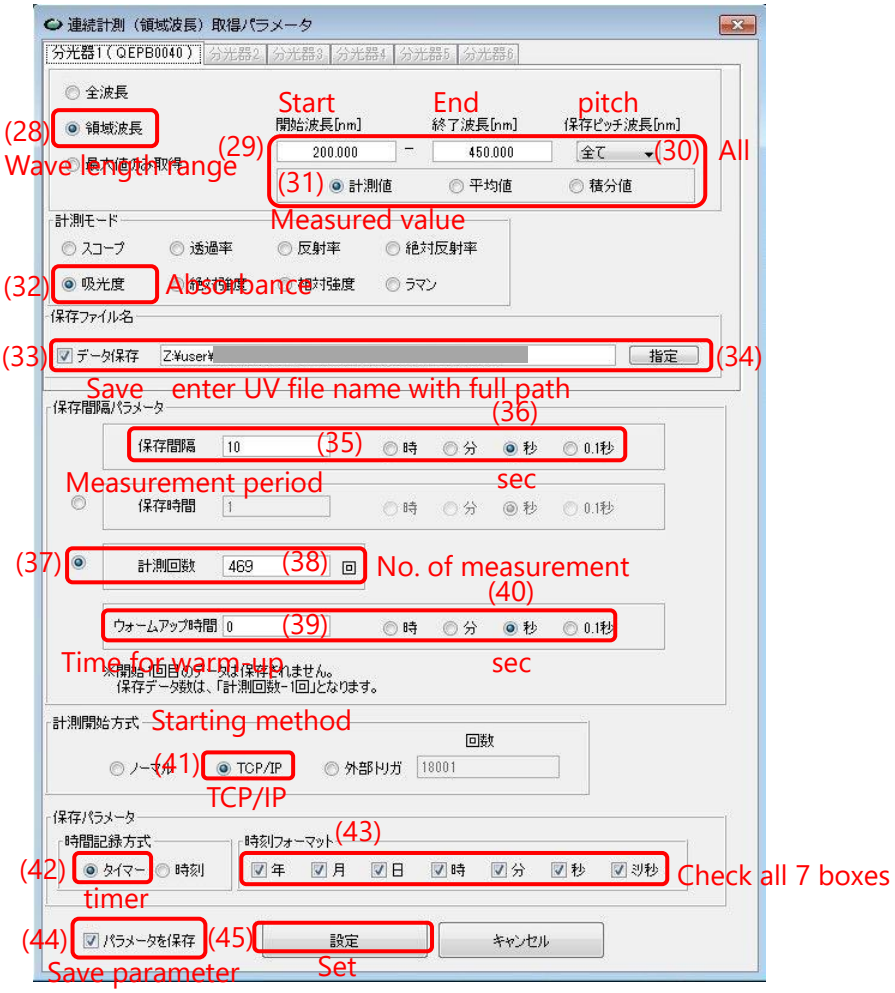
Click Menu icon (26), and select [連続計測(領域波長)]: serial measurements (spectra) (forth line) (27).

[連続計測(領域波長)]: serial measurements (spectra) window will open.





Set parameters in the window as described below.



[領域波長]: wave length range (28)　200 – 450 nm (29)

[保存ピッチ]: pitch / [全て]: all (30), Check [計測値]: measured value (31)

[計測モード]: mode / [吸光度]: absorbance (32)

[保存ファイル名]: file name / check [データ保存] :save data (33), [enter UV file name (***_UV)] (34)

[保存間隔パラメータ]: parameters for measurement period section

[保存間隔]: measurement period / [10] sec [[5]sec @BL-15A2] (35) and check [秒]:sec (36).

Check [計測回数]: No. of measurement (37), enter [469] [[637] @BL-15A2] (38)

[ウォームアップ時間]: time for warm-up / [0] (39) and check [秒]: sec (40).

[計測開始方式]: starting method / Check [TCP/IP] (41).

[保存パラメータ]: other parameters for saving section

Check [タイマー] : Timer (42) and Check all 7 boxes in [時刻フォーマット]: time format (43)

Check [パラメータを保存]: save parameters check box (44).

Click [設定]: Set icon (45).

Notification window will open. Click OK.

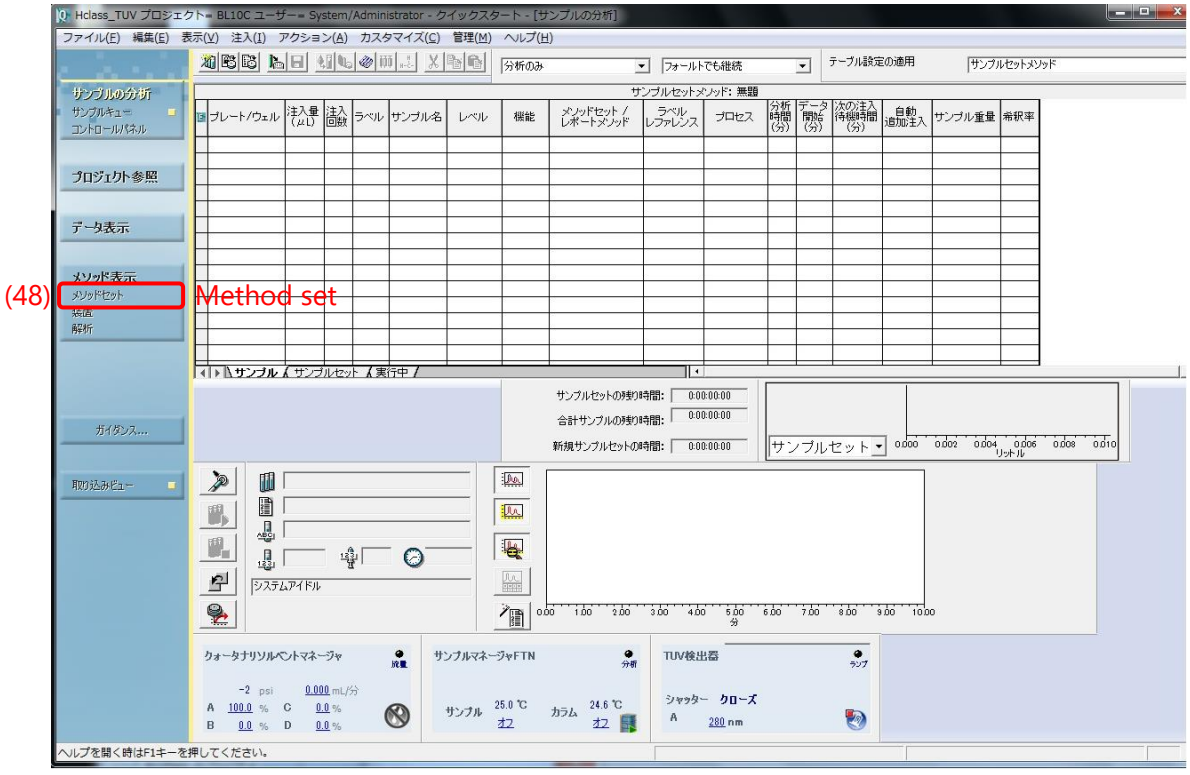
4D-2 preparation for serial measurement

Click [stop] icon (red icon) (46) to stop [live mode], and click [serial measurement] icon (clock icon) (47) to start waiting state for the start signal from PILUTAS.

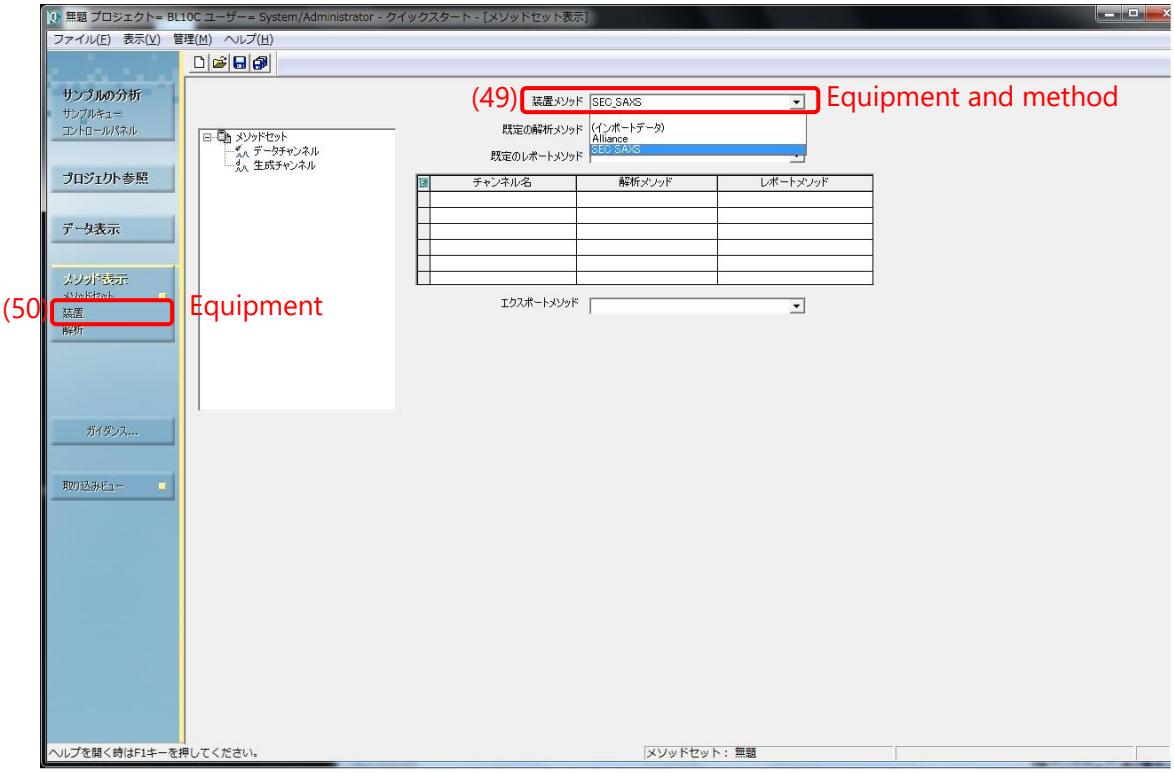
4E Setting of UPLC

4E-1 Opening [SEC-SAXS] method

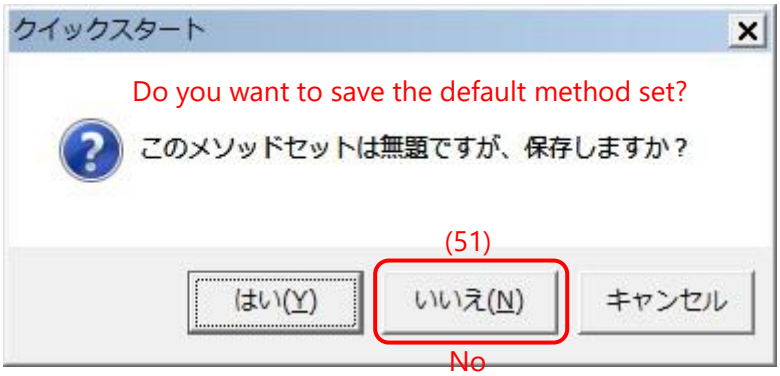
Click [メソッドセット]: Method set (48) in the navigation bar (left side of UPLC control software).



Select [SEC-SAXS] (49) in the [装置メソッド]: equipment and method, and click [装置]: equipment (50) in the navigation bar.



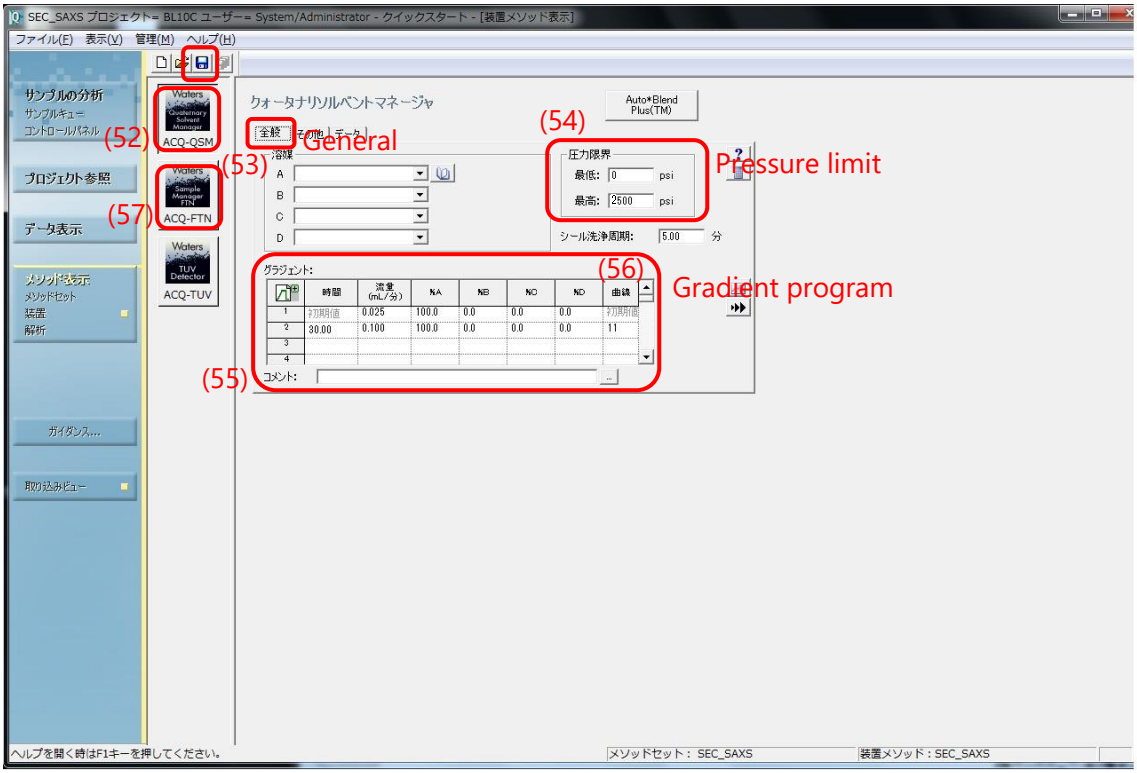
[クイックスタート]: quick start window will open, select [いいえ]: No (51) .

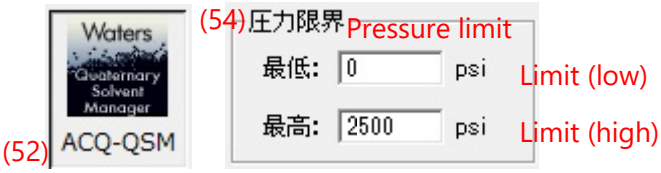


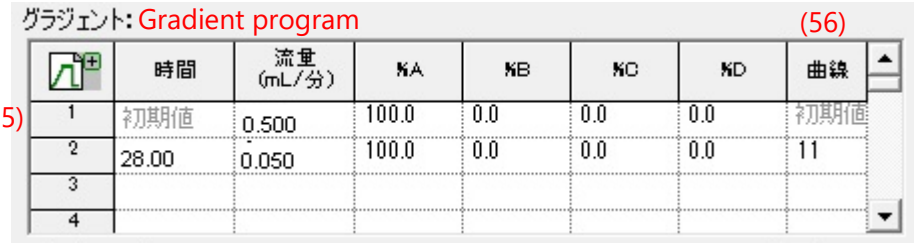
4E-2　Setting flow rate of [SEC-SAXS] method

Click [ACQ-QSM] icon (52), and move to [全般]: general tab (53).

Set [圧力限界]: pressure limit (54) (for example [最高]: max 2500 psi for Superdex200 increase 10/300)　*Pressure limit depends on the column, check manual or catalogue.







Set flow rate (55) in the [グラジェント]: gradient section table.

	時間 time	流量 Flow rate	%A	%B	%C	%D	曲線 curve
1	初期値 initial	0.5	100.0	0.0	0.0	0.0	初期値 initial
2	28	0.05 0.1 [BL-15A2]	100.0	0.0	0.0	0.0	11

Set [11] in the curve field (56). ([11] means that the flow rate will change ASAP.)

You can increase the steps by right-clicking on the table.

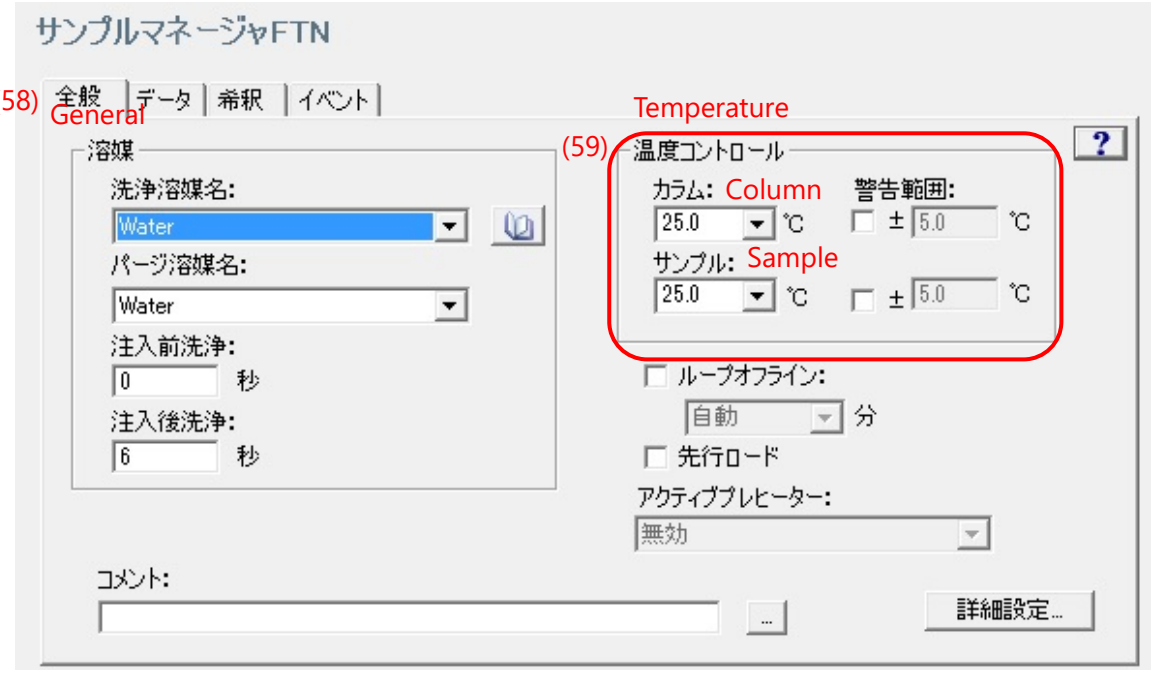
4E-3　Setting temperatures of [SEC-SAXS] method

Click [ACQ-FTN] icon (57), and move to [全般]: general tab (58).

Set column and sample temperatures (59) in [温度コントロール]: temp section.

Do not check [警告範囲]: warning range section.

(57)

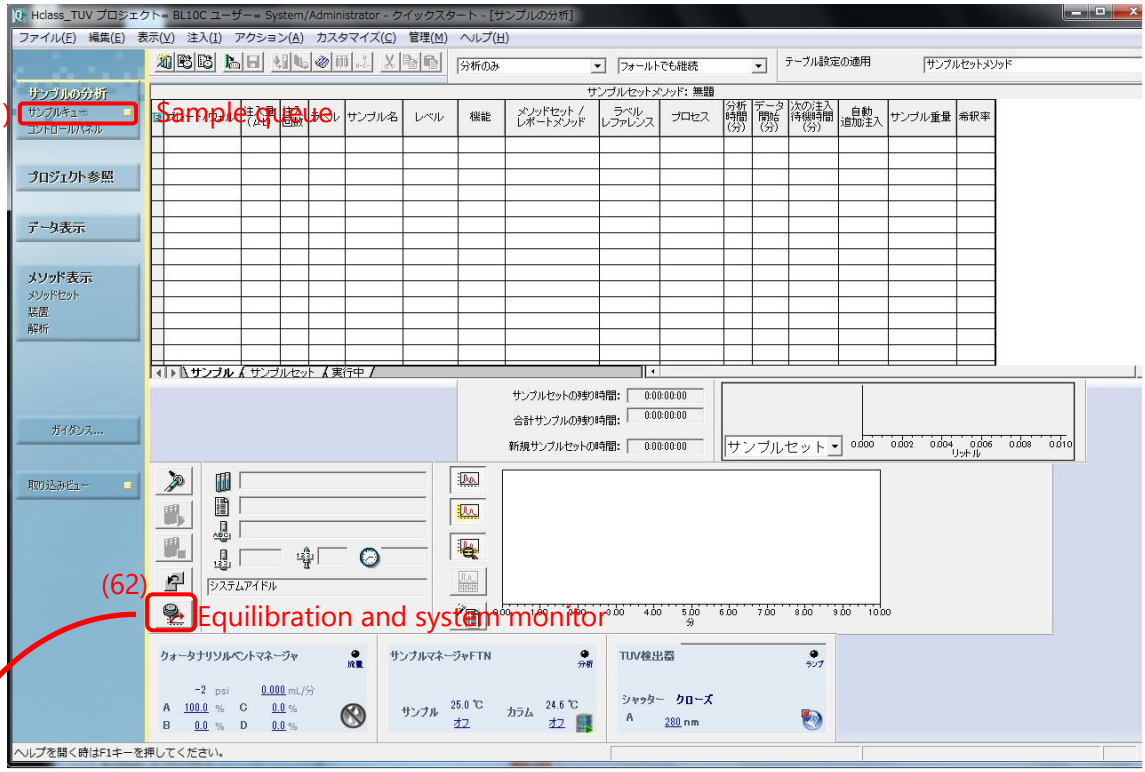


4E-4　Saving modified [SEC-SAXS] method

Click save icon (60) to save modified method.

※To save parameters is needed to run modified method. If you do not click save icon, UPLC will not work proper method.

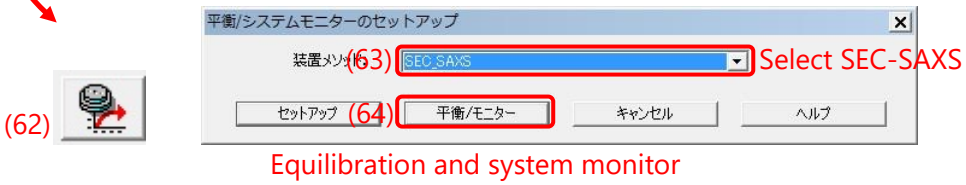
Click [サンプルキュー]: sample queue (61) in the navigation bar.



4E-5　　Starting equilibration and system monitor

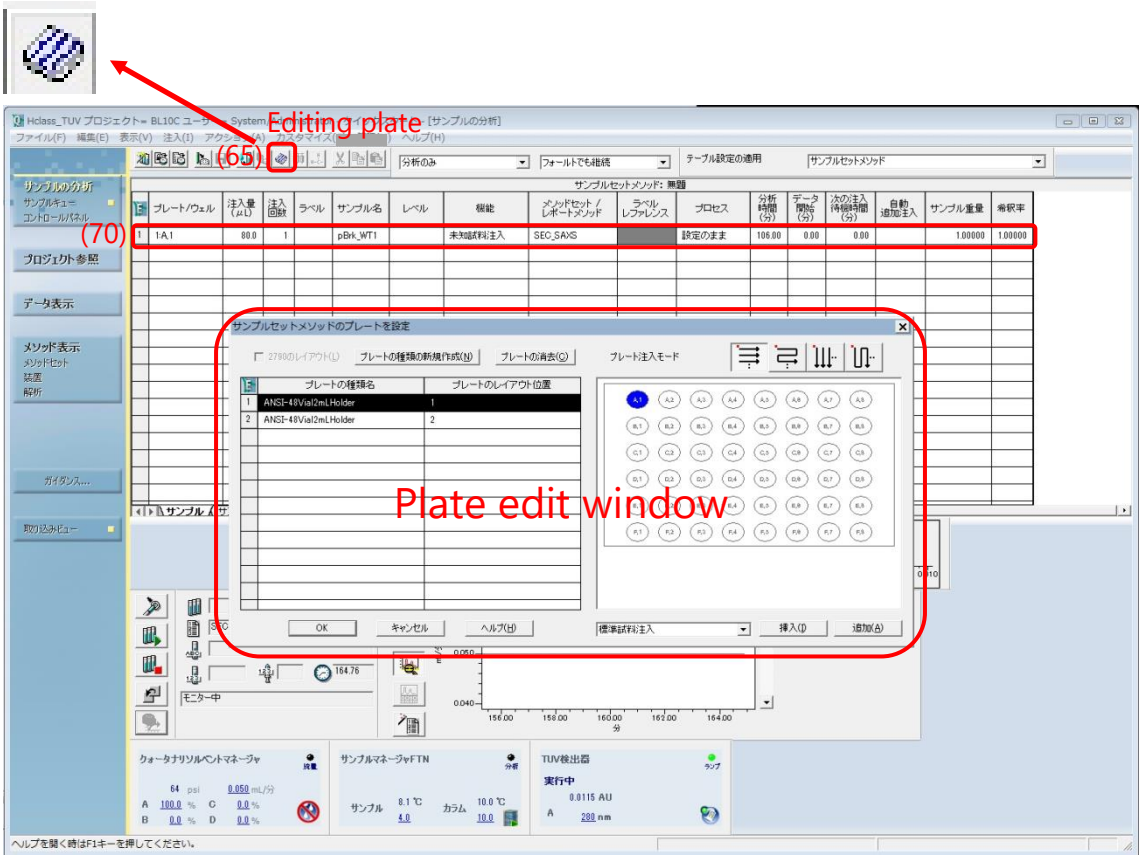
Click [平衡/システムモニター]: equilibration and system monitor icon (62). [平衡/システムモニター]: equilibration and system monitor window will open.

Select [SEC_SAXS] (63) in the [装置メソッド]: equipment and method box, and click [平衡/モニター]: equilibration and system monitor button (64). Buffer will flow as the initial values of [SEC-SAXS] method (Sample injection does not start.)



4E-6　　Setting up plate parameters

Click [プレート編集]: editing plate icon (65), The plate edit window will open.



In the plate edit window, select [ANSI-48 Vial 2ml Holder 1] (66) in the left side, and select [A1] position (67) in the right side.

Click [挿入]: insert (68) and click [OK] (69).

4E-7 Setting up injection parameters

Set parameters for the injection of UPLC (70).

Plate/well position　No. of injection　Function

Injection volume　Sample name　Method set/Report method

	プレート/ウェル (71)	注入量 (μL) (72)	注入回数 (73)	ラベル	サンプル名 (74)	レベル	機能 (75)	メソッドセット / レポートメソッド (76)
1	1:A,1	100	1		test		未知試料注入	SEC_SAXS

Unknown sample　SEC-SAXS

Analysis time

ラベル レファレンス	プロセス	分析時間 (分) (77)	データ開始 (分)	次の注入待機時間 (分)	自動追加注入	サンプル重量	希釈率
	設定のまま	78	0.00	0.00		1.00000	1.00000

[プレート/ウェル]: plate and well position / [1:A,1] (plate 1, A-1 position) (71) input have completed at 4E-6

[注入量]: injection volume (ul) / enter injection volume (max 250 ul) (72).

[注入回数]: No. of injection / [1] (73)

[サンプル名]: sample name / [enter sample name] (74).

[機能]: function / select [未知試料注入]: injection of unknown sample (second line) (75).

[メソッドセット/レポートメソッド]: method set and report method / select [SEC_SAXS] (76).

[分析時間]: analysis time (min) / enter [78] [[53] @BL-15A2] (77)

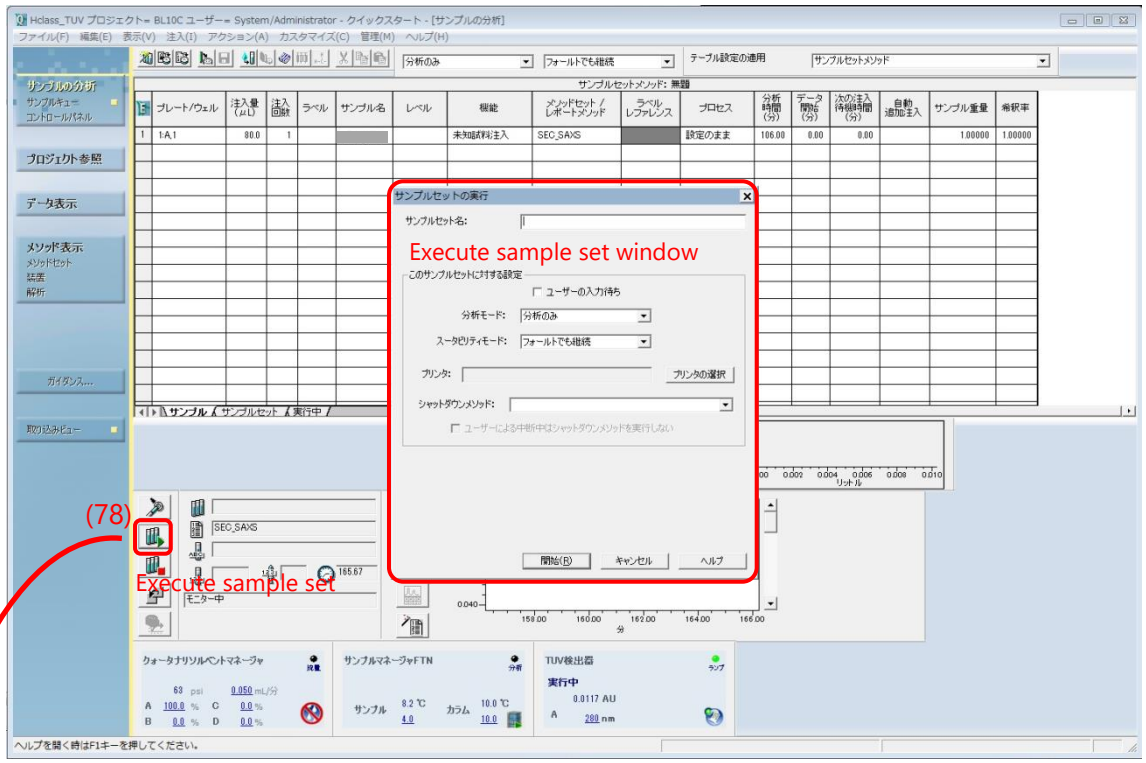
4F Execution of SEC-SAXS

4F-1 Start of the UPLC injection

Prepare SAXS flow cell and sample vial.

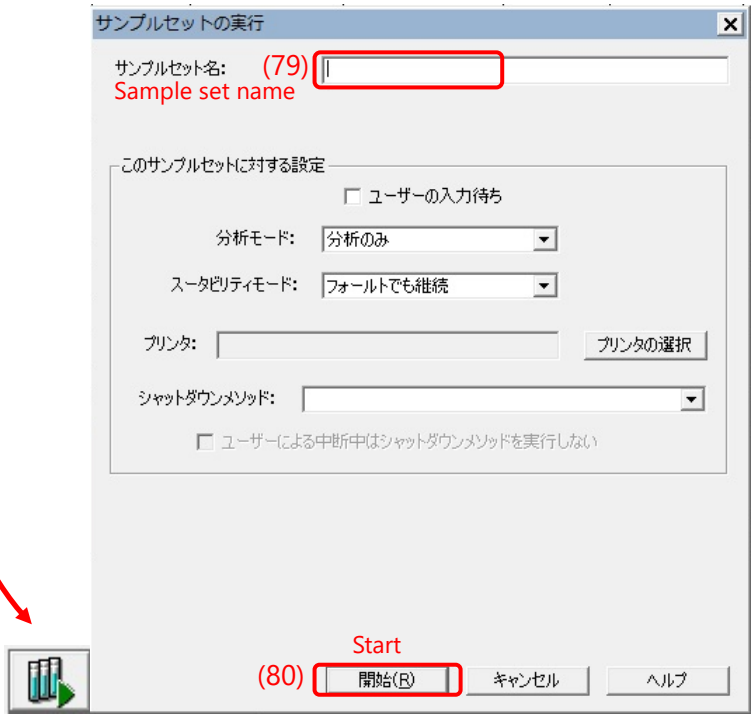
Close the experimental hatch and open DSS.

Click [execute sample set] icon (test tubes icon) (78), [execute sample set] window will open.



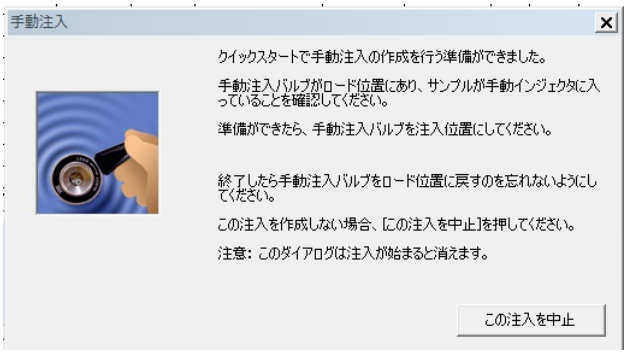
Enter [サンプルセット名]: sample set name (79) (You can set the same as the sample name and the sample set name).

Click [開始]: Start icon (80) to start injection process.



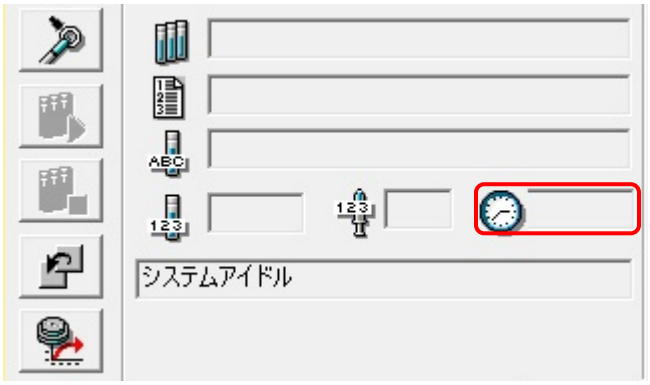
4F-2 Waiting time for the UPLC injection

The injection window will open. Just wait for the injection (waiting period:160 sec for 250 ul injection)



4F-3 Checking start of UPLC injection

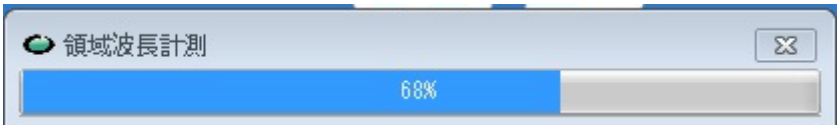
Check that the injection of UPLC start (the timer starts to count up.)



4F-4 Starting PILATUS measurement

Immediately after starting UPLC, click [Run] in PILATUS measurement software to start PILATUS. While PILATUS is running, a part of PILATUS measurement soft window will become pink in color and measured images will be displayed on the PILATUS image viewer software window. (You need to change PILATUS image viewer software to [measurement mode].)

Confirm the serial measurement of UV spectrophotometer start simultaneously, while the serial measurement of UV spectrophotometer, the progress bar window will open and update spectra on OPwave+EXT-K window.



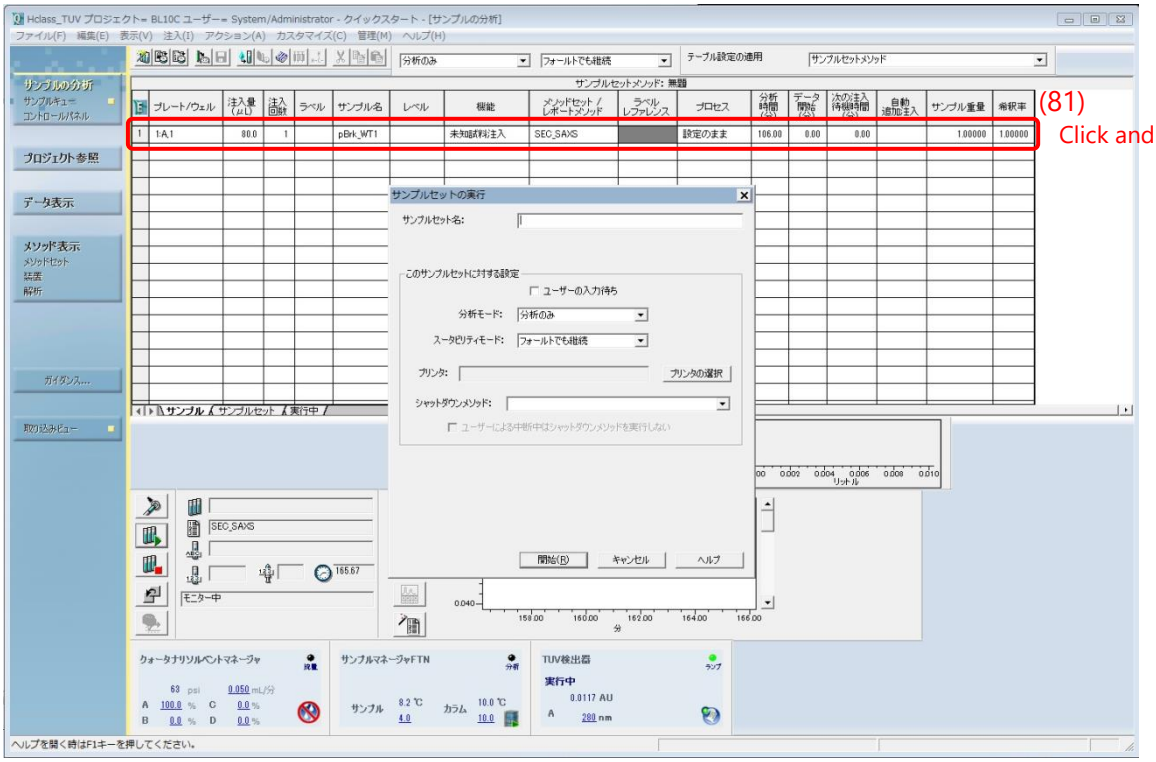
4F-5 Preparation for next measurement

After the SEC-SAXS measurement finish, increase the flow rate to re-equilibrate the column

on UPLC control software (because UPLC continue the flow at final programmed condition.) Right-click the parameter line in the sample set tab and select delete (81).

CAUTION: executed sample parameters does not deleted automatically. If you forget to delete them, the same methods will be performed on next run.

And Notification window will open on OPwave+EXT-K, click OK and re-start [live mode] again by clicking [green start icon] (82) of OPwave+EXT-K.



5 Synchronization mode

In this mode, PILUTUS and UV spectrophotometer will start automatically after the start of UPLC.

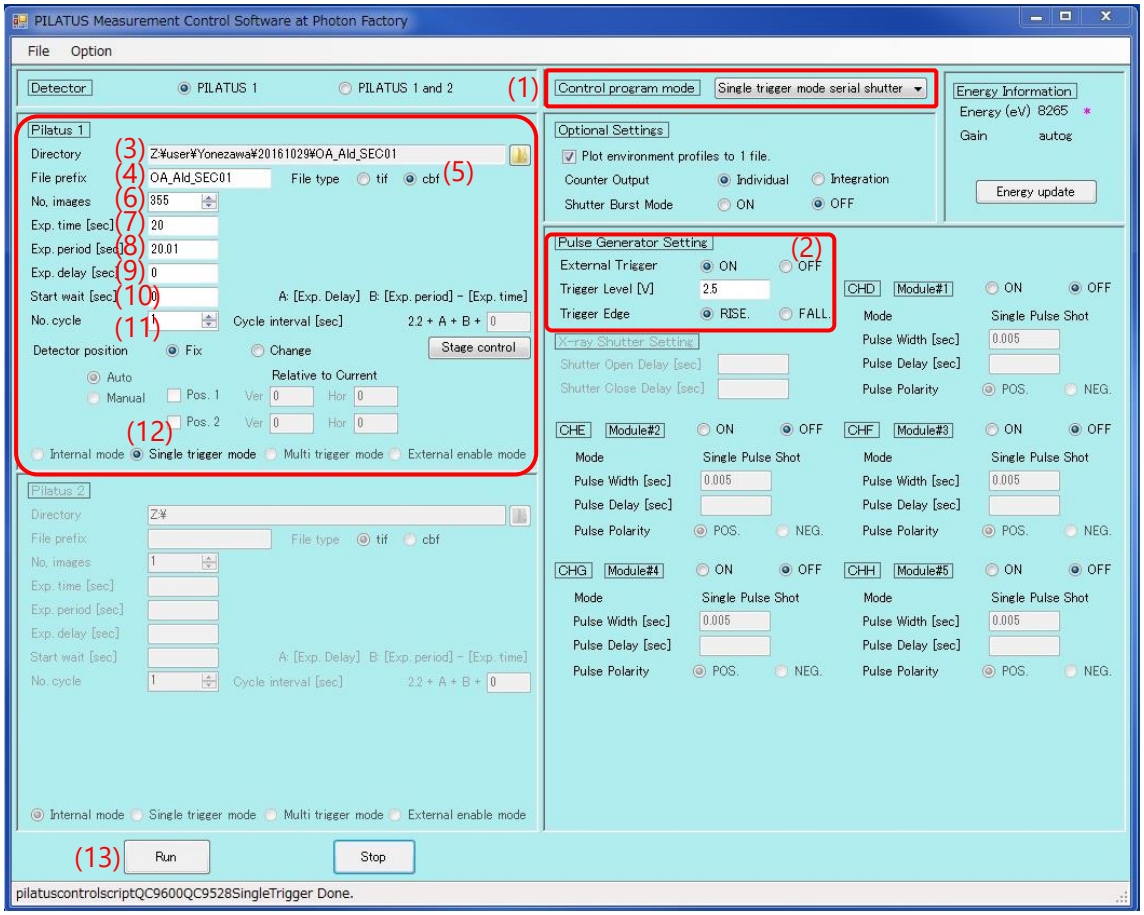
5A SAXS measurement of the background (buffer)

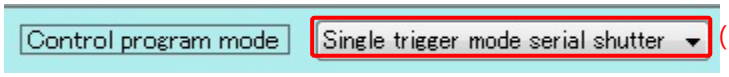
5A-1 Lowering the flow rate

After the equilibration of the column, lower the flow rate to 0.05 ml/min (same as measurement at peak fraction.) 0.1 ml/min@BL-15A2) and wait several minute until the pressure will be stable.

5A-2 Confirmation [Control program mode] of PILATUS Measurement Control Software

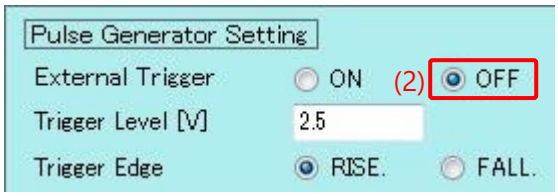
Set [Single trigger mode serial shutter] in [Control program mode] of PILATUS Measurement Control to start PILATUS by external trigger from UPLC





5A-3 Changing external trigger mode

Check External Trigger [OFF] (2) in Pulse Generator Setting Area.



5A-4 Measurement of background images

Measure SAXS image of background (buffer) (for example 20sec x 15 times@BL-10C, 10sec x 15 times@BL-15A2.)

Set parameters in [PILATUS Measurement Control Software] as described below.

Directory [enter your folder] (3)

File Prefix [enter your file prefix] (4)

File type [select cbf or tif] (5) (If you want to process the data by other than SAngler, select tif.)

No.images [15] (6)

Exp. Time [20] @BL-10C default, [[10] @BL-15A2] (7)

Exp.period [20.01] @BL-10C default, [[10.01] @BL-15A2] (8)

Exp. delay [0] (9)

Start wait [0] (For SEC-SAXS experiment) (10)

No.cycle [1] (For SEC-SAXS experiment) (11)

Check [Single trigger mode] (12).

Close the experimental hatch to open X-ray shutter before start.

Click [Run] (13) to start measurement.

5A-5 Restoring the change of external trigger mode

After measurement of background, Check External Trigger [ON] in Pulse Generator Setting Area.

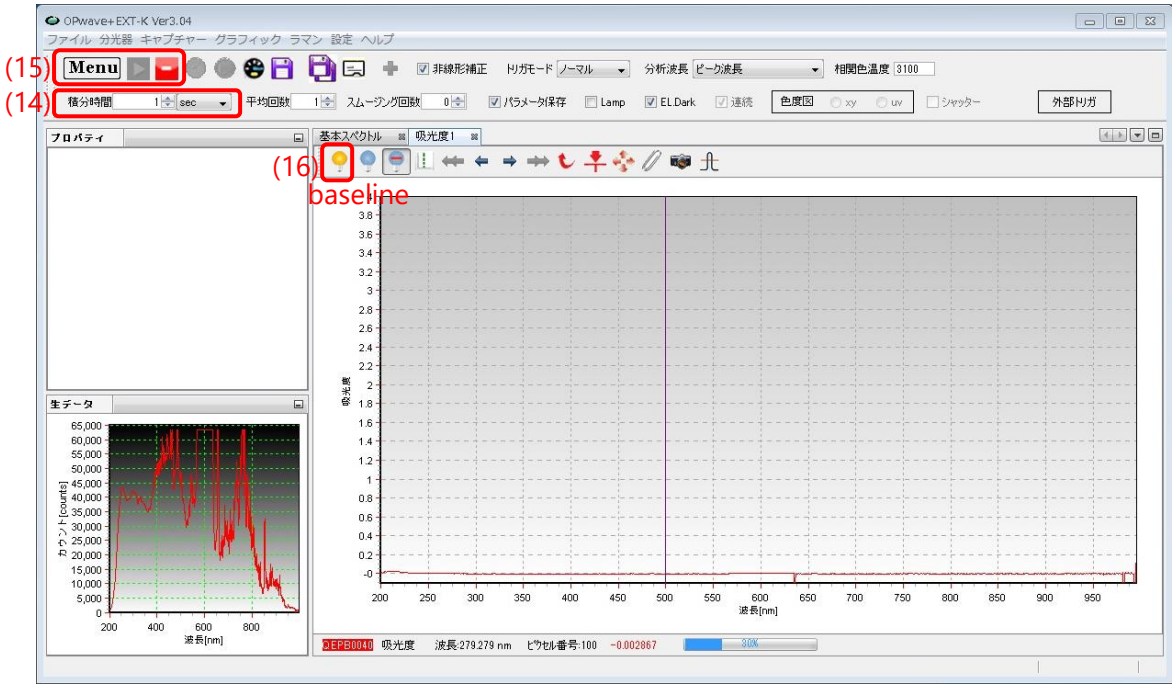
5B Setting of UV spectrophotometer software.

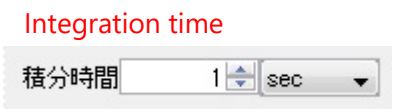
5B-1 Initial setting of UV spectrophotometer software

Confirm that [積分時間]: integration period is [1sec] (14) in OPwave+EXT-K (UV

spectrophotometer software). If different period is set, the re-adjustment of the UV spectrophotometer is needed, see p.5 of OPwave manual or Appendix4.

Confirm that OPwave+EXT-K is [live mode].
If the icon next to menu icon is grey and the spectra is renewed every sec, OPwave+EXT-K is set to [live mode].
If the icon next to menu icon is green, Click that green icon (15), and set OPwave+EXT-K to [live mode].





5B-2 measurement of reference spectra with UV spectrophotometer

Check SAXS flow cell position and no air bubble in the flow path. And check the flow rate is 0.05 ml/min@BL-10C [0.1 ml/min@BI-15A2]

And then click the yellow bulb icon (16) to get reference spectra.

Measurement Example

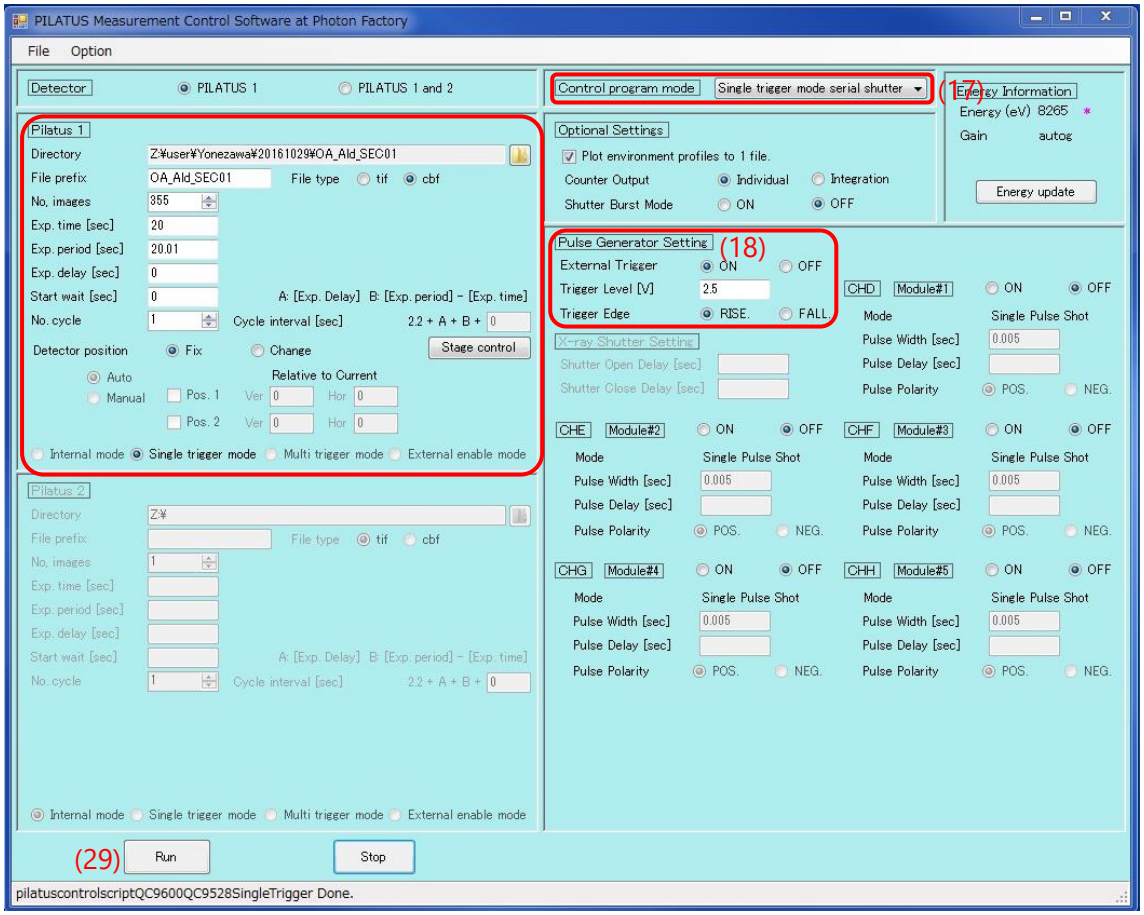
Column : Superdex200 increased 10/300 (CV: 24ml)
Flow rate 0.5 ml/min @0~14 ml(0~28 min) and 0.05 ml/min @14~16.5 ml(28~78min) (0.1 ml/min @14~16.5 ml(28~53min) @BL-15A2).
(Measure peak fractions with slower flow rate.)

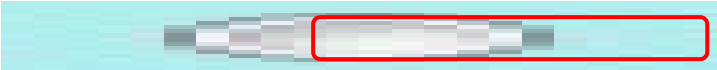
SAXS (expose 20sec/image, exp period 20.01sec)、
UV measurement period is set to 10sec as default (twice frequency as X-ray).
(@BL-10C default value. @BL-15A2 SAXS expose 10sec/image, period 10.01sec, UV 5sec)
Total number of measurements are
78 x 60 /20.01 = 234 (SAXS) [53 x 60 / 10.01 = 318 @BL-15A2]
78 x 60 /10 +1 = 469 (UV) [53 x 60 / 5 +1 = 637 @BL-15A2]

5C Setting parameters of PILATUS measurement control software

5C-1 Confirmation [Control program mode] of PILATUS Measurement Control Software

Set [Single trigger mode serial shutter] (17) in [Control program mode] of PILATUS Measurement Control.





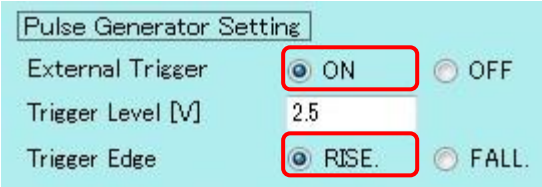
5C-2 Confirmation parameters of Pulse generator setting

Confirm parameters (18) in Pulse Generator Setting Area.

External Trigger [ON]

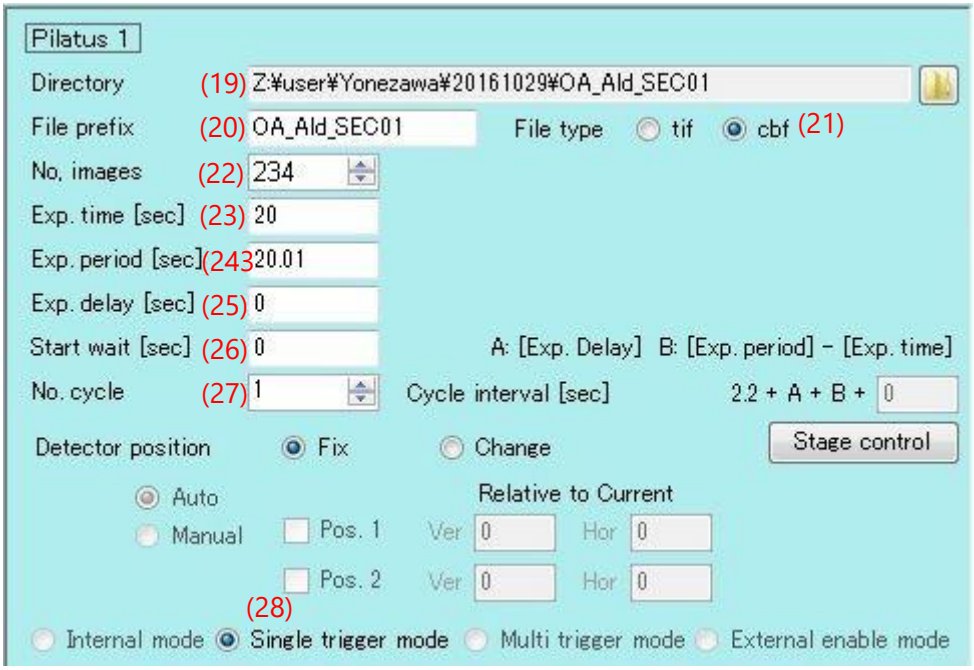
Trigger Level [2.5]

Trigger Edge [RIZE]



5C-3 Setting up of PILATAS measurement parameters

Set parameters in PILATUS Measurement Control Software as described below.



Directory [enter your folder] (19)

File Prefix [enter your file prefix] (20)

File type [select cbf or tif] (21) (If you want to process the data by other than SAngler, select tif.)

No.images [234] @BL-10C [[318] @BL-15A2] (22)

Exp. Time [20] @BL-10C default, [[10] @BL-15A2] (23)

Exp.period [20.01] @BL-10C default, [[10.01] @BL-15A2] (24)

Exp. delay [0] (25)

Start wait [0] (For SEC-SAXS experiment) (26)

No.cycle [1] (For SEC-SAXSexperiment) (27)

Check [Single trigger mode] (28).

5C-4 Starting PILATUS measurement

Click [Run] icon (29), PILATUS starts waiting state for the start signal from UPLC. A part of PILATUS measurement software window will become pink in color.

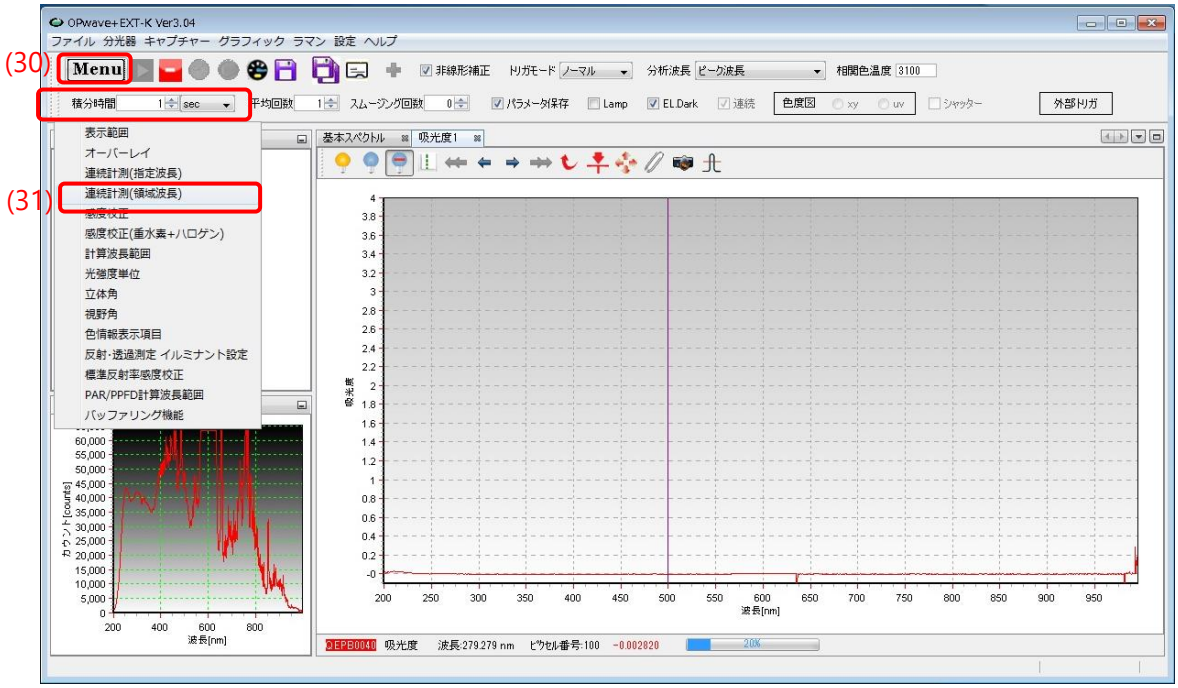
5D Setting parameters of OPwave+EXT-K and Pulse Generator Control for UV spectrophotometer.

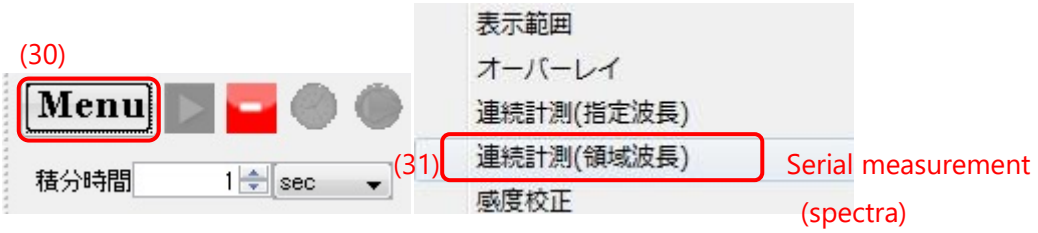
Both of UV spectrophotometer control software (OPwave+EXT-K) and Pulse Generator Control for UV spectrophotometer are needed for synchronization mode.

5D-1 Setting parameters of OPwave+EXT-K

Click Menu icon (30), and select [連続計測(領域波長)]: serial measurements (spectra) (forth line) (31).

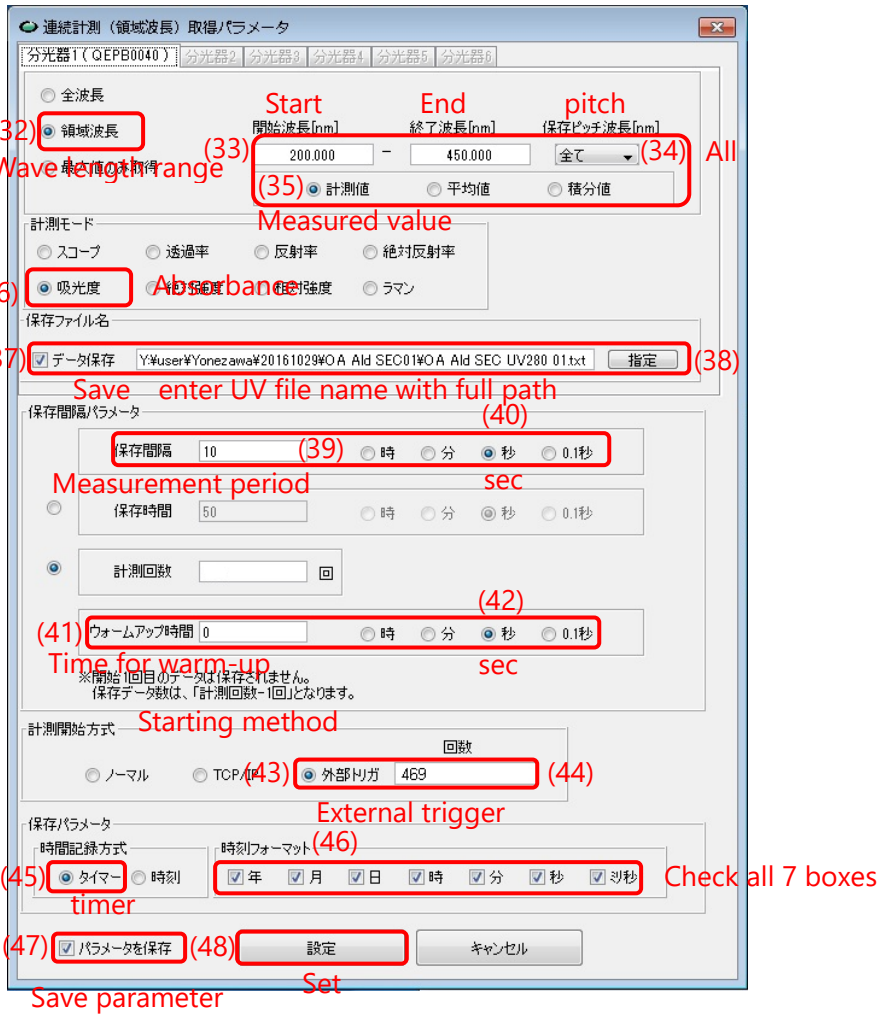
[連続計測(領域波長)]: serial measurements (spectra) window will open.





5D-2 Setting parameters of OPwave+EXT-K

Set parameters in the window as described below.



[領域波長]: wave length range (32)　200 – 450 nm (33)

[保存ピッチ]: pitch / [全て]: all (34), Check [計測値]: measured value (35)

[計測モード]: mode / [吸光度]: absorbance (36)

[保存ファイル名]: file name / check [データ保存] :save data (37), [enter UV file name (***_UV)] (38)

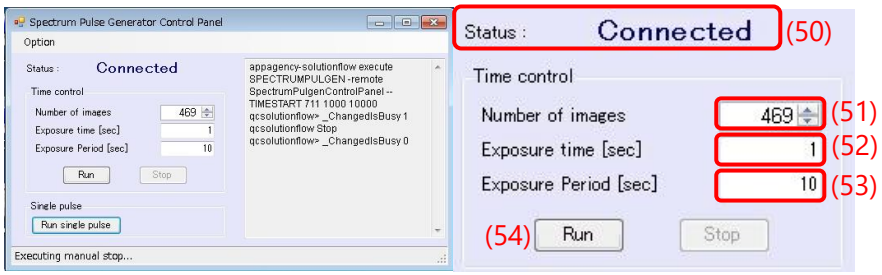
[保存間隔パラメータ]: parameters for measurement period section
[保存間隔]: measurement period / [10] sec [[5] sec @BL-15A2] (39) and check [秒]: sec (40).
[ウォームアップ時間]: time for warm-up / [0] (41) and check [秒]: sec (42).
[計測開始方式]: starting method / Check [外部トリガ]: external trigger (43) and enter [469] [[637] @BL-15A2] (44).
[保存パラメータ]: other parameters for saving section
Check [タイマー] : Timer (45) and Check all 7 boxes in [時刻フォーマット]: time format (46)
Check [パラメータを保存] : save parameters check box (47).
Click [設定]: Set icon (48).
Notification window will open. Click [OK].

5D-3　　Starting UV spectrophotometer measurement

Click [stop icon] (red icon) to stop [live mode], and click [serial measurement icon] (clock icon) to start waiting state for the start signal from PILUTAS.

5D-4　Setting Pulse Generator Control software for UV spectrophotometer

Confirm the status of [Spectrum Pulse Generator Control Panel] is [Connected] (50).
Enter the parameters in “Time Control”.

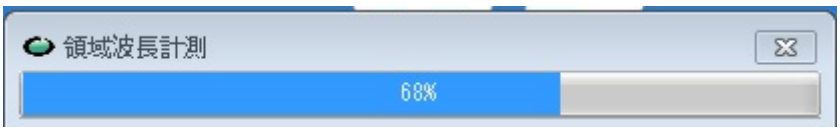


<u>These parameters should be the same values which are entered in OPwave+EXT-K, see 5D-2.</u>
Number of images [469]：measurement number　[[637] @BL-15A2] (51)
Exposure time [sec] [1]：integration period　（The default value is 1sec）(52)
Exposure Period [sec] [10]：measurement interval [[5] sec @BL-15A2] (53)

5D-5　　Starting Pulse Generator Control software for UV spectrophotometer

Click Run icon (54) to start waiting state for the start signal from PILATUS.
The status of [Spectrum Pulse Generator Control Panel] will be changed to [Running] and a part of the window will changed to pink in color.
While the serial measurement of UV spectrophotometer, the progress bar window will open.

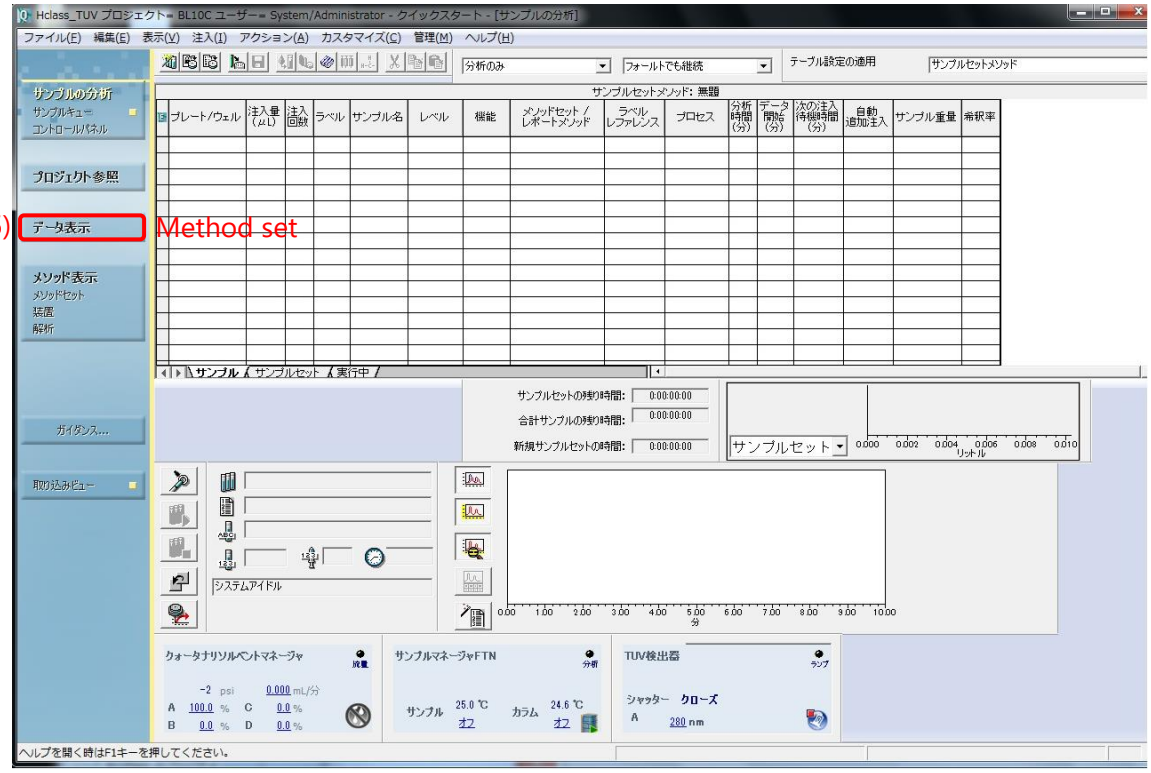
(Before the injection of UPLC, the progress value is kept 0 %.)



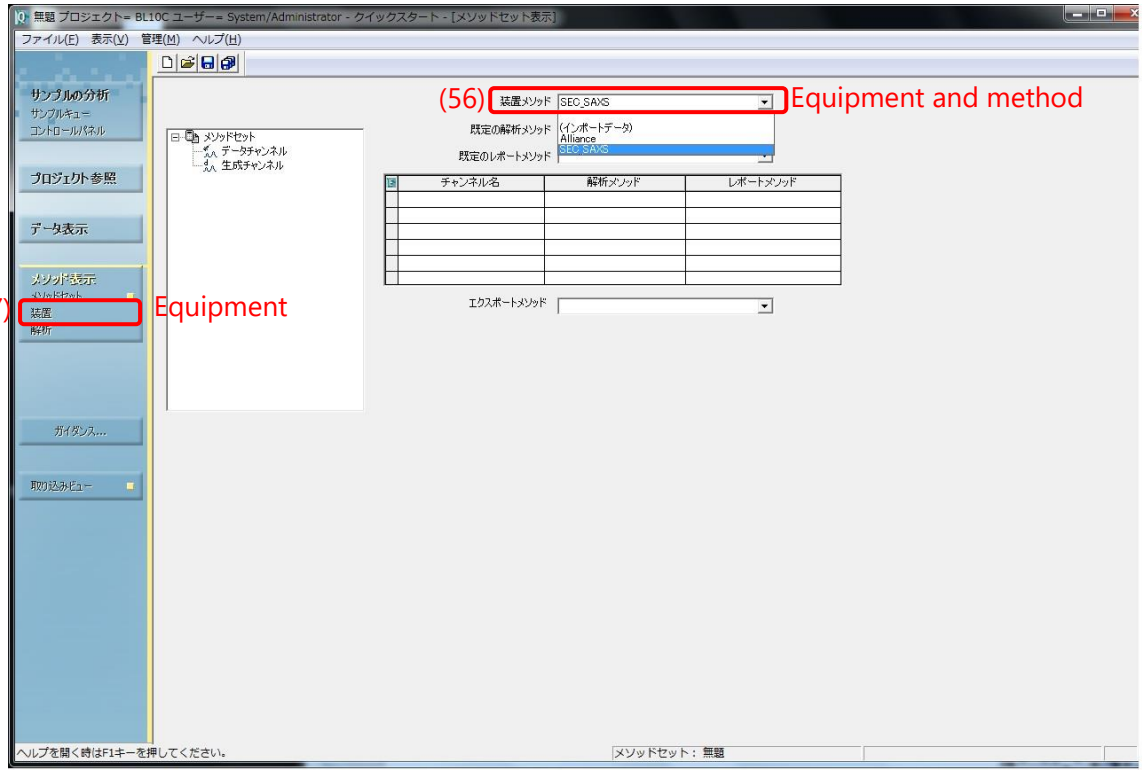
5E Setting of UPLC

5E-1 Opening [SEC-SAXS] method

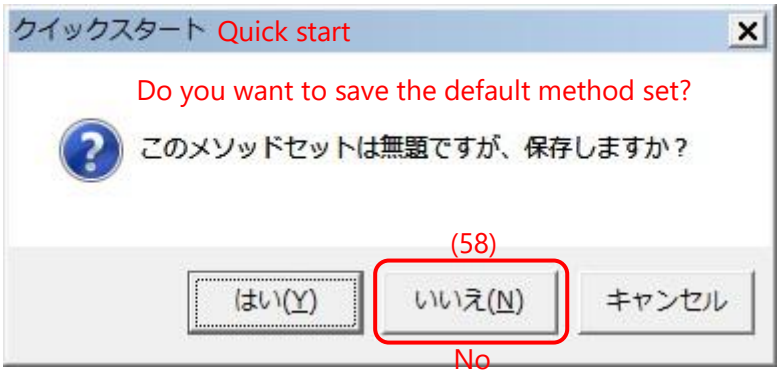
Click [メソッドセット]: Method set (55) in the navigation bar (left side of UPLC control software).



Select [SEC-SAXS] (56) in the [装置メソッド]: equipment and method, and click [装置]: equipment (57) in the navigation bar.



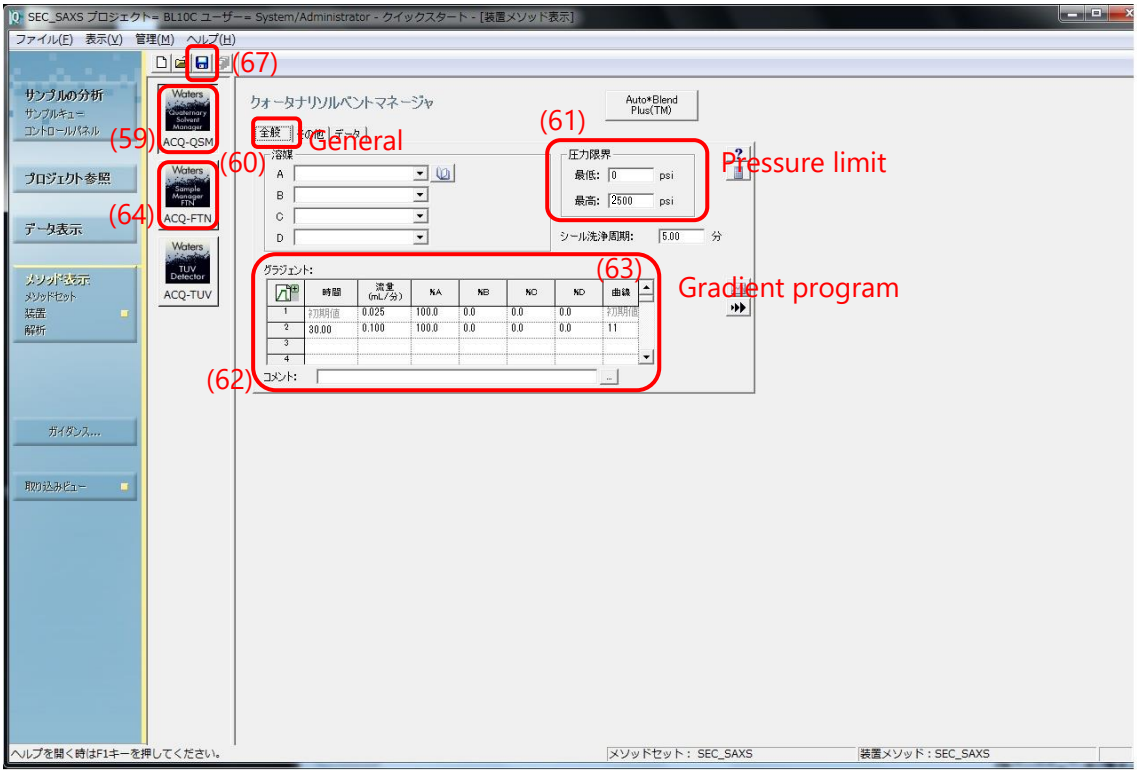
[クイックスタート]: quick start window will open, select [いいえ]: No (58).

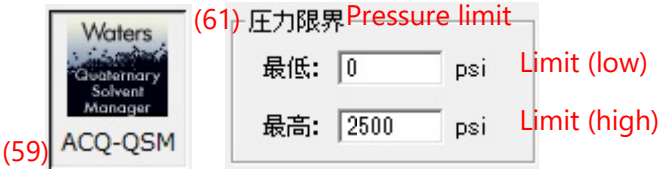


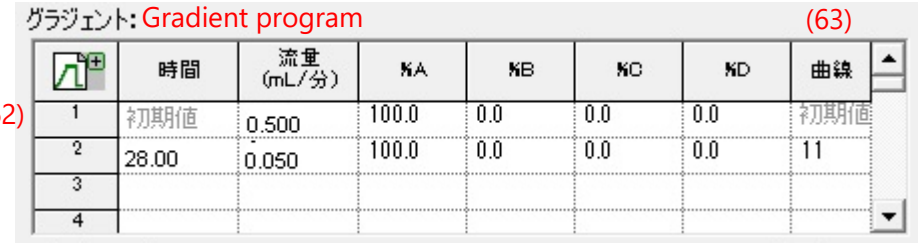
5E-2　Setting flow rate of [SEC-SAXS] method

Click [ACQ-QSM] icon (59), and move to [全般]: general tab (60).

Set [圧力限界]: pressure limit (61) (for example [最高]: max 2500 psi for Superdex200 increase 10/300)　*Pressure limit depends on the column, check manual or catalogue.







	時間	流量 (mL/分)	%A	%B	%C	%D	曲線
1	初期値	0.500	100.0	0.0	0.0	0.0	初期値
2	28.00	0.050	100.0	0.0	0.0	0.0	11
3							
4							

Set flowrate (62) in the [グラジェント]: gradient section table.

	時間 time	流量 Flow rate	%A	%B	%C	%D	曲線 curve
1	初期値 initial	0.5	100.0	0.0	0.0	0.0	初期値 initial
2	28	0.05 0.1 [BL-15A2]	100.0	0.0	0.0	0.0	11

Set [11] in the curve field (63). ([11] means that the flowrate will change ASAP.)

You can increase the steps by right-clicking on the table.

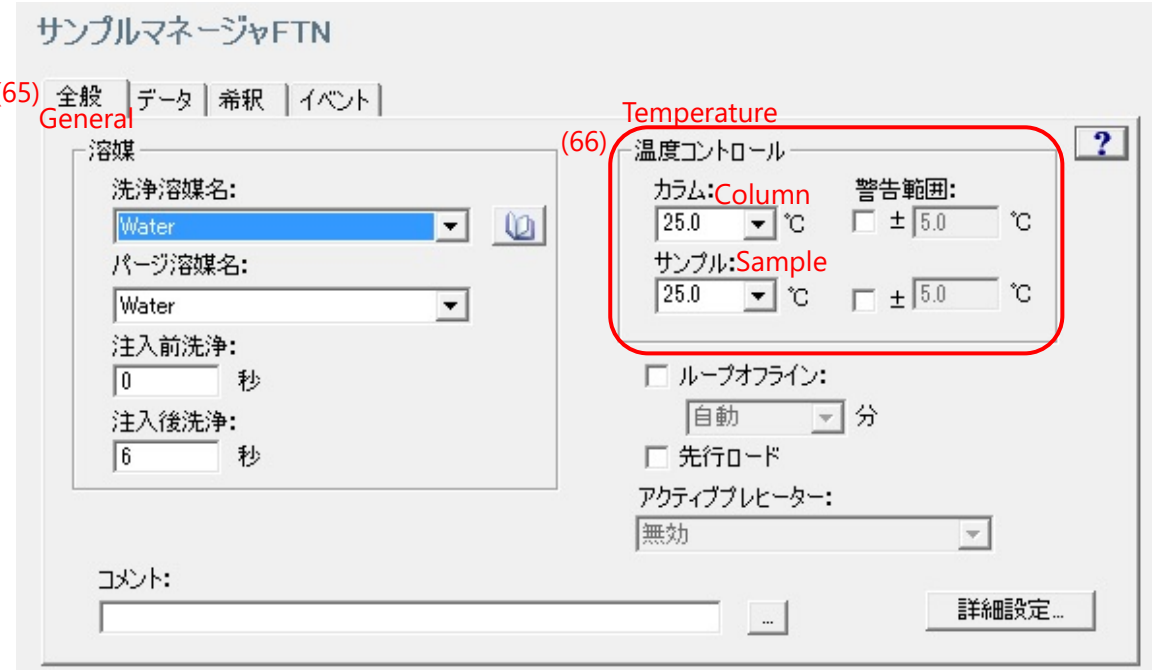
5E-3　　Setting temperatures of [SEC-SAXS] method

Click [ACQ-FTN] icon (64), and move to [全般]: general tab (65).

Set column and sample temperatures (66) in [温度コントロール]: temp section.

Do not check [警告範囲]: warning range section.

(64)



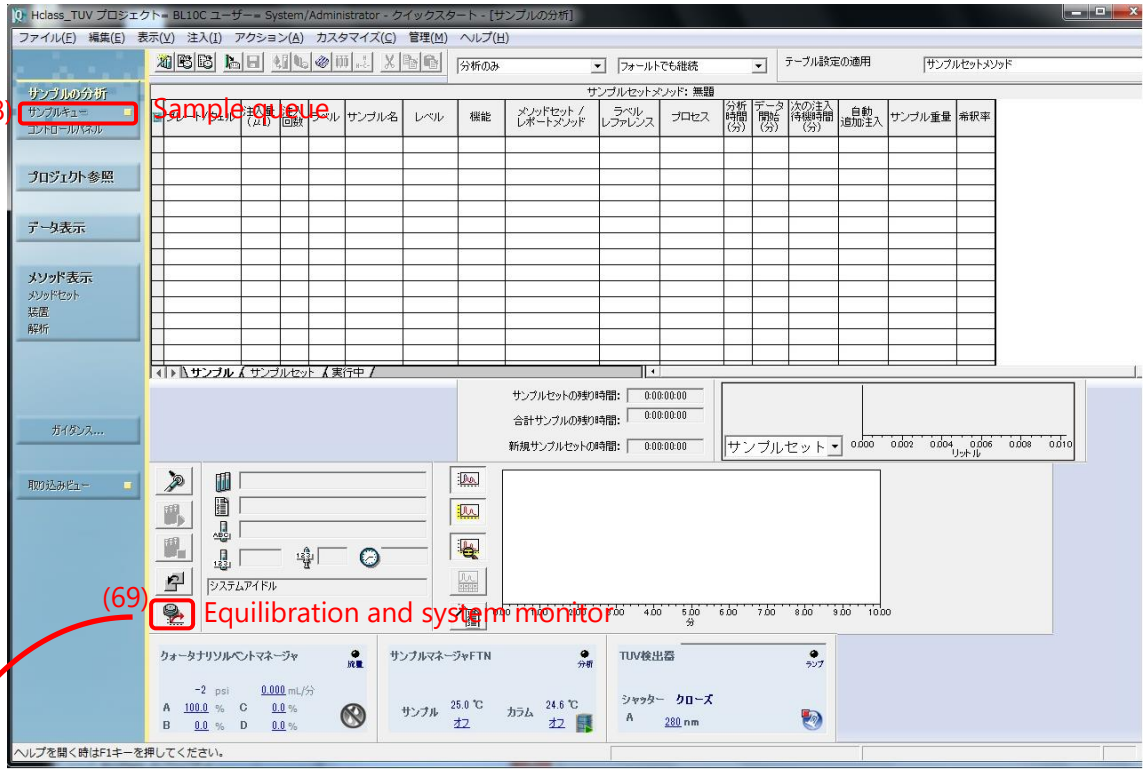
5E-4　　Saving modified [SEC-SAXS] method

Click save icon (67) to save modified method.

※To save parameters is needed to run modified method. If you do not click save icon, UPLC will not work proper method.

(67)

Click [サンプルキュー]: sample queue (68) in the navigation bar.



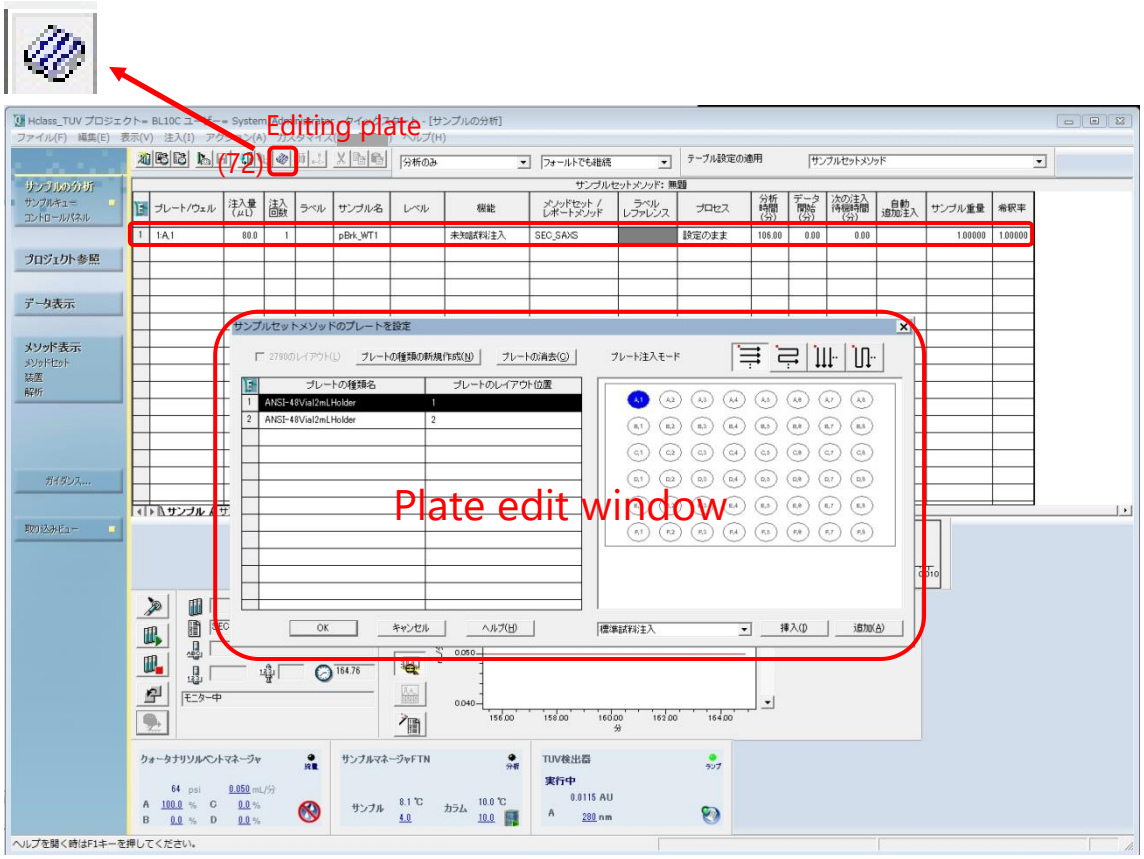
5E-5　　Starting equilibration and system monitor

Click [平衡/システムモニター]: equilibration and system monitor icon (69). [平衡/システムモニター]: equilibration and system monitor window will open.

Select [SEC_SAXS] (70) in the [装置メソッド]: equipment and method box, and click [平衡/モニター]: equilibration and system monitor button (71). Buffer will flow as the initial values of [SEC-SAXS] method (Sample injection does not start.)

5E-6　　Setting up plate parameters

Click [プレート編集]: editing plate icon (72), The plate edit window will open.



In the plate edit window, select [ANSI-48 Vial 2ml Holder 1] (73) in the left side, and select [A1] position (74) in the right side.

Click [挿入]: insert (75) and click [OK] (76).

5E-7 Setting up injection parameters

Set parameters for the injection of UPLC.

Plate/well position　No. of injection　Function

Injection volume　Sample name　Method set/Report method

	プレート/ウェル (77)	注入量 (μl) (78)	注入回数 (79)	ラベル	サンプル名 (80)	レベル	機能 (81)	メソッドセット / レポートメソッド (82)
1	1:A,1	100	1		test		未知試料注入	SEC_SAXS

Unknown sample　SEC-SAXS

Analysis time

ラベル レファレンス	プロセス	分析時間 (分) (83)	データ開始 (分)	次の注入待機時間 (分)	自動追加注入	サンプル重量	希釈率
	設定のまま	78	0.00	0.00		1.00000	1.00000

[プレート/ウェル]: plate and well position / [1:A,1] (plate 1, A-1 position) (77), input have completed at 5E-6

[注入量]: injection volume (ul) / [enter injection volume (max 250 ul)] (78).

[注入回数]: No. of injection / [1] (79)

[サンプル名]: sample name / [enter sample name] (80).

[機能]: function / select [未知試料注入]: injection of unknown sample (second line) (81).

[メソッドセット/レポートメソッド]: method set and report method / select [SEC_SAXS] (82).

[分析時間]: analysis time (min) / enter [78] [[53] @BL-15A2] (83).

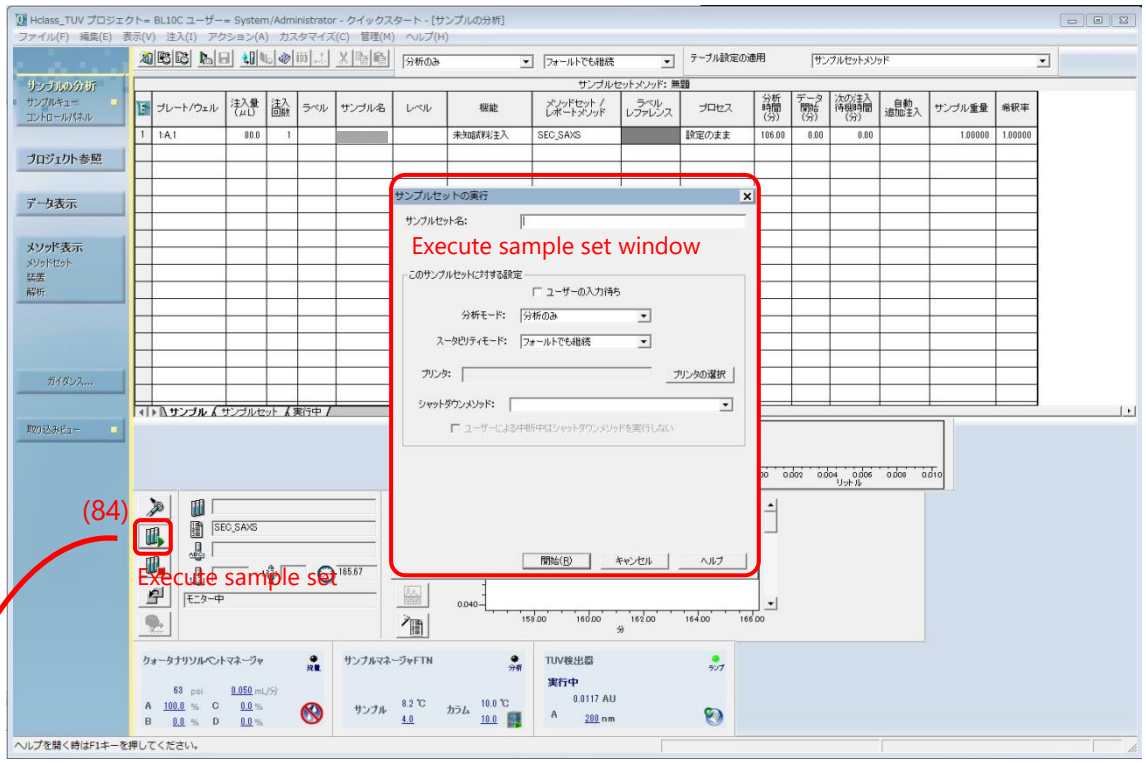
5F Execution of SEC-SAXS

5F-1 Start of the UPLC injection

Prepare SAXS flow cell and sample vial.

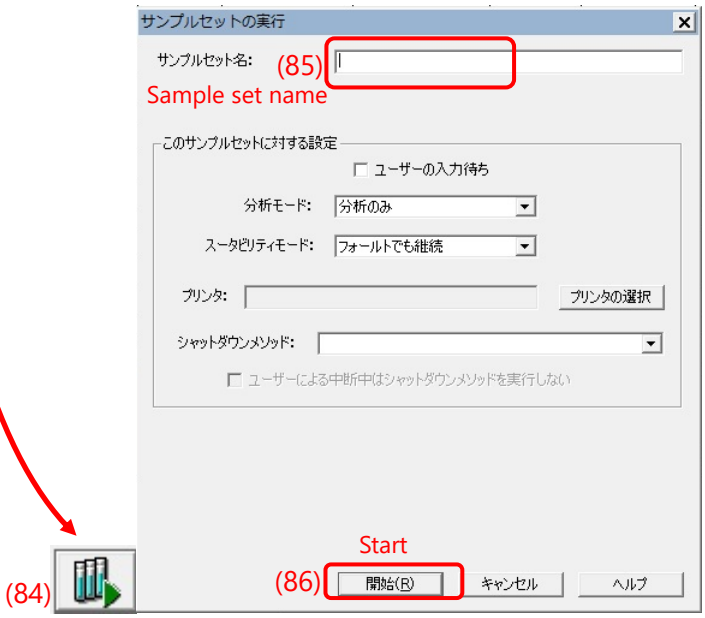
Close the experimental hatch and open DSS.

Click [execute sample set] icon (test tubes icon) (84), [execute sample set] window will open.



Enter [サンプルセット名]: sample set name (You can set the same as the sample name and the sample set name).

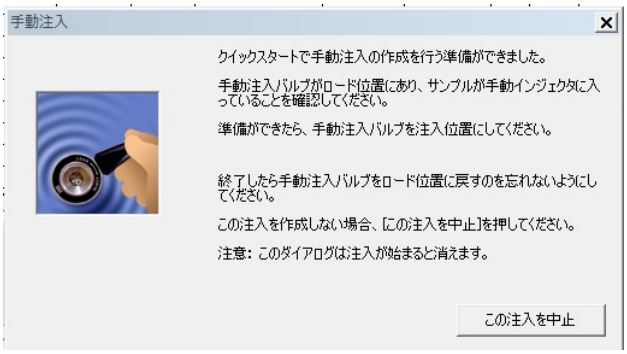
Click [開始]: Start icon to start injection process.



5F-2 Waiting time for the UPLC injection

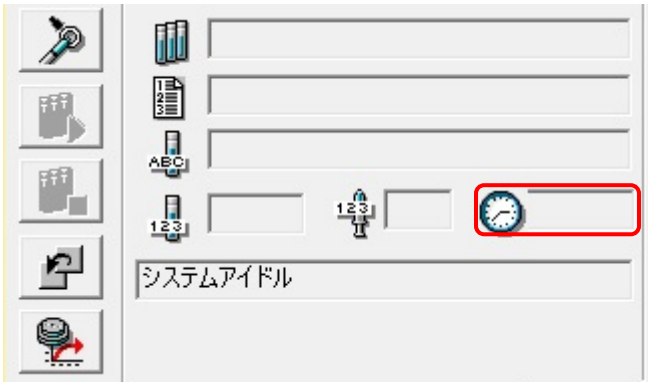
The injection window will open. Just wait for the injection (waiting period:160 sec for 250 ul

injection)



5F-3 Checking start of UPLC injection

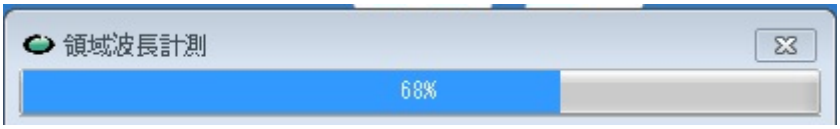
Check that the injection of UPLC start (the timer starts to count up.)



Both PILUTUS and UV spectrophotometer will start simultaneously.

While PILATUS is running, a part of PILATUS measurement soft window will become pink in color and measured images will be displayed on the PILATUS image viewer software window. (You need to change PILATUS image viewer software to [measurement mode].)

Confirm the serial measurement of UV spectrophotometer is working. While the serial measurement of UV spectrophotometer, the progress bar window will proceed and update spectra on OPwave+EXT-K window.



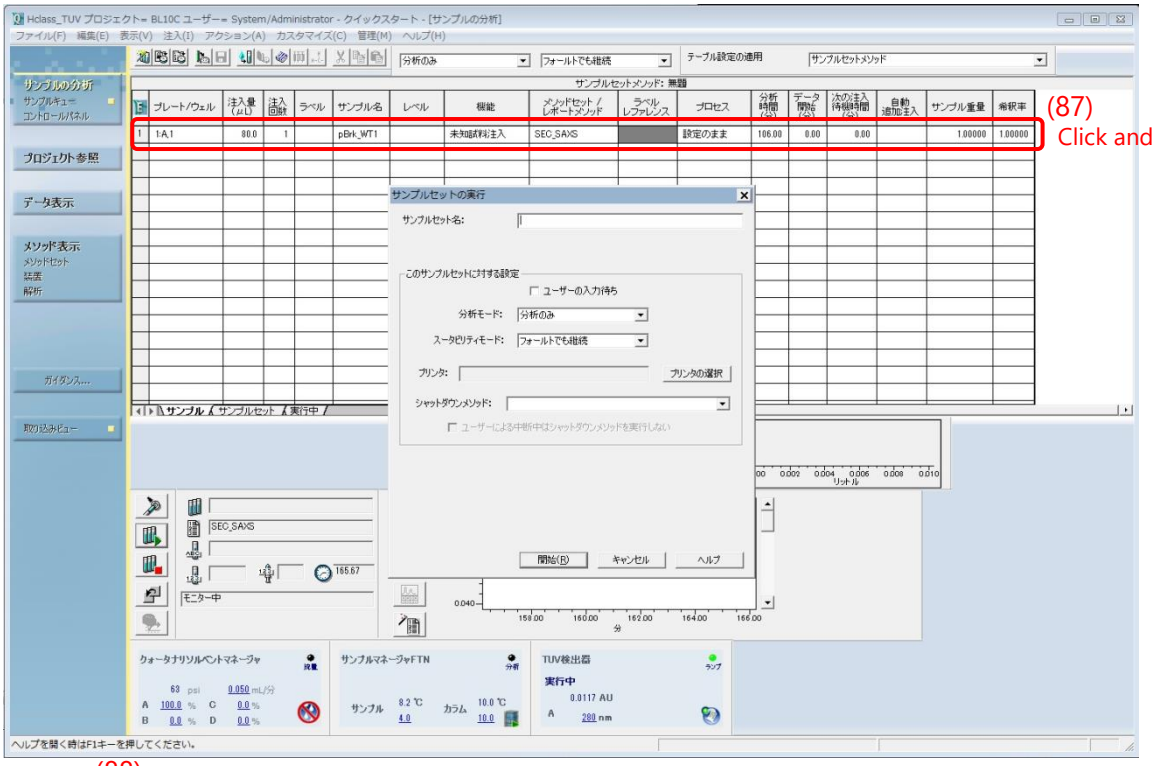
5F-4 Preparation for next measurement

After the SEC-SAXS measurement finish, increase the flow rate to re-equilibrate the column on UPLC control software (because UPLC continue the flow at final programmed condition.)

Right-click the parameter line in the sample set tab and select delete (87).

CAUTION: executed sample parameters does not deleted automatically. If you forget to delete them, the same methods will be performed on next run.

And Notification window will open on OPwave+EXT-K, click OK and re-start [live mode] again by clicking [green start icon] (88) of OPwave+EXT-K.



6 Procedure after SEC-SAXS experiment

6-1

Perform step 1A-1 ~5 with MilliQ [A line]. And then flow MilliQ [A line] at low flow rate (0.05ml/min with Superdex 200 increase 10/300).

*Keep eye on the remaining amount of MilliQ.

6-2 Disposal of waste fluid (waste cup and brown glass bottle), pipette tips and vials.

- Waste fluid: plastic container for hydrous organic solvents [in case of buffer without heavy metal ions] @ Chemistry Lab. of PF.
  The primary and secondary washing solution must be collected in the plastic container. And then rinse cup and bottle with tap water and place them on the shelf @ Biophysics & Molecular physiology Lab. of PF.

- Pipette tips and vial caps: Trash can for cap, plastics @ Biophysics & Molecular physiology Lab. of PF.

- Vial: Trash can for glassware @ Biophysics & Molecular physiology Lab. of PF.

6-3

Wash the flow cell with peristaltic pump and dry it with air pump.

Rinse the unions and ferrules with MilliQ.

If you find a stain on the window of the flow cell, note it on the log book.

FAQ

1) An error occurred on UPLC control software.

See Appendix 1, and eliminate the cause of trouble.

2) The UV drift on OPwave-EXT-K is large.

The UV baseline is changed by flow rate. Therefore UV base line must be set at the same flow rate as the sample elution period.

In case of unstable and large UV drift, try to change SAXS flow cell.

3) OPWave-EXT-K is frozen or stacked.

See Appendix4, and restart OPWave-EXT-K and perform initial setting.

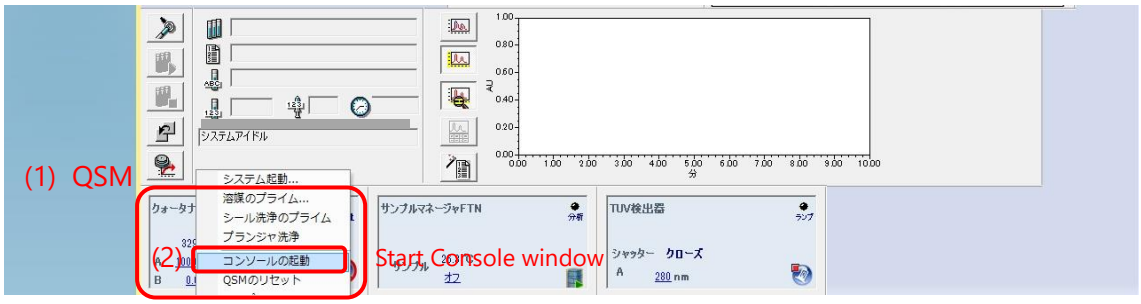
付録 1 Error of UPLC (Empower3)

1

An UPLC error occurs, the error message appears at the display part of Empower3 window.

2

Right-click the QSM (クォータナリーソルベントマネージャ:quaternary solvent manager) part (1) of the direct control panel of the UPLC control software and select [コンソールの起動]: Start Console Window (fifth line) (2).

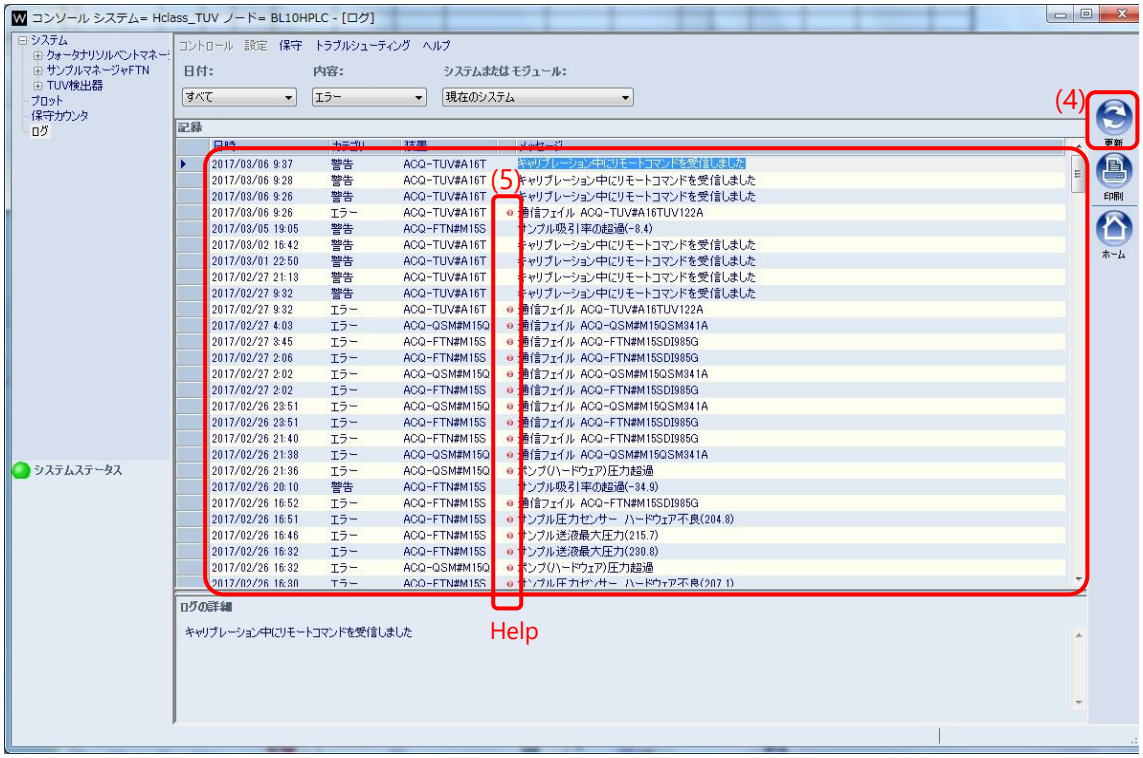


3

The Console Window will open, Click [ログ]: log (3) in the left side.

4

Check the latest error message. If the error message at the proper time stamp doesn't appear, click [更新]:update icon (right side) (4).



5

Click red circle icon (5) on the left side of the error message to open the help window, and follow that help message to remove the cause of error.

If the error message about pressure appears, check these points.

- Use only fresh filtrated buffer (less than 0.2 um filter).
- Use only supernatant after centrifugation (15k rpm, 10 min) or filtrated solution (less than 0.2 um filter) as the sample.
- Remove and install the connections between PEEK tube and Column.
  Set 5mm length PEEK tubing is projected from the tip of the fitting, connect the fitting and the column by hand.
- Perform longer needle wash (30 sec) and many times of FTN priming (15 sec of washing solvent line and 25 times of purging syringe) , see 1A-8,9.
- Set the drawing sample rate lower, see Appendix 5.

6

Errors and Warnings occurred until now.

Warning (UPLC doesn't stop)：サンプル吸引率の超過[aspirated sample volume is low.]

警告	ACQ-FTN#M15S	サンプル吸引率の超過(-34.9)

The aspirated sample volume is less than the programmed sample volume.
(You can continue the program. [It is better to check how much volume of sample is injected from the remaining volume of sample vial. Or you can interrupt the program (re-equilibration is needed for the next experiment.)

The particle in sample or buffer may be responsible for this warning.

- The sample solution must be supernatant (15krpm, < 10min) or filtered (0.2 um).
- Perform longer washing needle of FTN (60sec), see 1A-7.
- Perform longer priming of FTN [15] sec in [洗浄溶媒]: washing solvent line and [25] times in [パージ溶媒]: purging syringe, see 1A-8,9.
- Set the drawing sample rate lower, see Appendix 5.

Error：通信フェイル[Connection failed.]

エラー	ACQ-TUV#A16T	通信フェイル ACQ-TUV#A16TUV122A
エラー	ACQ-QSM#M15Q	通信フェイル ACQ-QSM#M15QSM341A
エラー	ACQ-FTN#M15S	通信フェイル ACQ-FTN#M15SDI985G

The connection between PC and UPLC is failed.

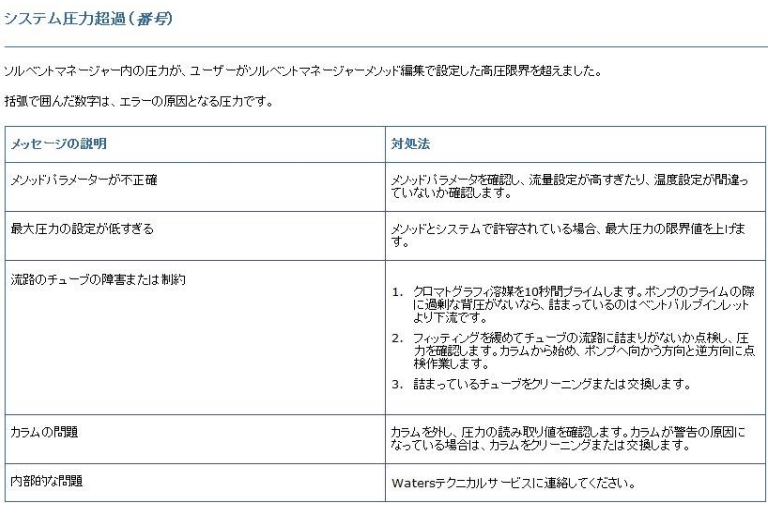
通信フェイル

メッセージの説明	解決法
設定上の問題：装置用 LAN と Ethernet 通信の設定が正しくない	1. Ethernet の設定を確認し、必要に応じて誤りを訂正します。 2. 問題があった装置をリセットします。 3. 警告が継続する場合は、システムまたはモジュールの電源を一旦切断してからまた入れ直します。 4. それでも警告が継続する場合は、Waters テクニカルサービスにご連絡ください。
電源に問題があるため、装置がワークステーションとネットワーク接続できない	1. 装置とワークステーションの電源とケーブルを確認します。 2. 装置とワークステーションの電源が入っていることを確認します。 3. 通信に問題があった装置をリセットします。 4. 警告が継続する場合は、システムまたはモジュールの電源を一旦切断してからまた入れ直します。 5. それでも警告が継続する場合は、Waters テクニカルサービスにご連絡ください。
Ethernet ケーブルが不適切または欠陥がある	シールドされた Ethernet ケーブルと交換します。

- Check LAN cables of the backside of UPLC equipment and PC in the experimental hatch.
- Reset the corrected equipment. [Right-click the area of the corrected equipment of direct control panel and select [リセット]: reset.]
- If the same error persists, reboot UPLC and PC system, see Appendix 3.

Error：システム圧力超過[Over pressure of system pressure]

System pressure is higher than pressure limit (high) that you set in Empower3.



システム圧力超過（番号）

ソルベントマネージャー内の圧力が、ユーザーがソルベントマネージャーメソッド編集で設定した高圧限界を超えました。

括弧で囲んだ数字は、エラーの原因となる圧力です。

メッセージの説明	対処法
メソッドパラメーターが不正確	メソッドパラメータを確認し、流量設定が高すぎたり、温度設定が間違っていないか確認します。
最大圧力の設定が低すぎる	メソッドとシステムで許容されている場合、最大圧力の限界値を上げます。
流路のチューブの障害または制約	1. クロマトグラフィ溶媒を10秒間プライムします。ポンプのプライムの際に過剰な背圧がないなら、詰まっているのはベントバルブインレットより下流です。 2. フィッティングを緩めてチューブの流路に詰まりがないか点検し、圧力を確認します。カラムから始め、ポンプへ向かう方向と逆方向に点検作業します。 3. 詰まっているチューブをクリーニングまたは交換します。
カラムの問題	カラムを外し、圧力の読み取り値を確認します。カラムが警告の原因になっている場合は、カラムをクリーニングまたは交換します。
内部的な問題	Watersテクニカルサービスに連絡してください。

- Check the setting values of the flow rate and the system pressure limit.
  Set the lower flow rate or higher pressure limit.
- Check the clogging of tubing or channel of the system.
  Perform priming [A] line for 0.2 min to check See 1A-4, 5. If the back pressure value is still high, the clogging is downstream from the pump. Search the clogging part with loosening the fitting one by one. The direction to search is from the column to the pump. If the clogging parts are identified, clean or change them.
- Check the clogging of the column.
  Confirm the pressure value without the column. If the column is clogged, clean the column or change the column.

Error：リークが検出されました[Leakage has been detected.]

エラー　　ACQ-QSM#M15Q　　リークが検出されました

The buffer is leaking.

リークが検出

メッセージの説明	溶液
リークセンサー内に液体がある	1. リークの原因を探し出して、モジュールを修理します。 2. センサーを取り外して乾かします。 3. リークセンサーリザーバの液体を廃棄して、リザーバを乾かします。 4. センサーを再度取り付けます。 5. ソルベントマネージャーをリセットします。 6. 警告が継続する場合は、電源をいったん切って、再度入れ直します。 7. それでも警告が継続する場合は、Watersテクニカルサービスに連絡してください。

- Open the doors of each equipment, and identify the leakage position.
- Remove and reinstall that connection. Clean the leakage sensor and the reservoir of leakage sensor (wiping and drying) and reset QSM.
- If the same error persists, reboot UPLC and PC system, see Appendix 3.

Error：Z 軸移動　ハードウェア不良[Hardware failure of Z axis movement of FTN.]

エラー　　ACQ-FTN#M15S　　Z軸移動 ハードウェア不良

Zp軸移動のハードウェア不良

メッセージの説明	溶液
ニードルがセンサーフラグを妨げています。	ニードルが正しく取り付けられ、フラグを妨げていないことを確認してください。
プレートの寸法が正しく設定されていないかANSIバージョンのプレートではありません	プレートの寸法が正しく設定され、ANSIバージョンのプレートが使用されていることを確認します。
Z軸のキャリブレーションが正しくありません	Z軸のキャリブレーションが正しいことを確認します。
間違ったプレートが挿入されています	正しいプレートを挿入する。
プレートの挿入が正しくありません	プレートが正しく挿入されたことを確認してください。
Z軸が何かにぶつかっています	サンプルマネージャーの電源を一度切って、再度入れます。
内部的な問題	それでも警告が継続する場合は、Watersテクニカルサービスに連絡してください。

- Set the sample plate properly in the FTN, turn off the power of FTN and turn on the power of FTN.
- If the same error persists, reboot UPLC and PC system, see Appendix 3.

Error：ニードルアーム移動　ハードウェア不良[Hardware failure of needle arm of FTN.]

エラー　　ACQ-FTN#M15S　　ニードルアーム移動 ハードウェア不良

R軸 移動のハードウェア不良

メッセージの説明	溶液
内部的な問題	1. サンプルマネージャーをリセットします。 2. 警告が継続する場合は、電源をいったん切って、再度入れ直します。 3. それでも警告が継続する場合は、Watersテクニカルサービスに連絡してください。

- Reset FTN.
- If the same error persists, turn off the power of FTN and turn on the power of FTN.
- If the same error persists again, reboot UPLC and PC system, see Appendix 3.

Error：サンプル送液最大圧力[Sample fluidics high pressure limit.]

エラー　ACQ-FTN#M15S　サンプル送液最大圧力(215.7)

エラー　ACQ-FTN#M15S　Sample fluidics high pressure limit (219.3)

サンプル流路系の最大圧力

値は、送液システムが最大圧力を超えたこと(50 psigより上)を示しています。

メッセージの説明	溶液
ニードルまたは内部バルブ出入り口の送液ラインに、詰まり、折れ曲がり、または破損などのシステム障害がある	▪ トランスデューサから下流までに詰まりがないか点検します。 ▪ チューブ(ニードル)の折れ曲がりや破損の有無を確認し、損傷したチューブを交換します。
内部的な問題	1. サンプルマネージャーをリセットします。 2. 警告が継続する場合は、電源をいったん切って、再度入れ直します。 3. それでも警告が継続する場合は、Watersテクニカルサービスに連絡してください。

- Perform longer washing needle of FTN (60sec), see 1A-7.
- Perform longer priming of FTN [15] sec in [洗浄溶媒]: washing solvent line and [25] times in [パージ溶媒]: purging syringe, see 1A-8,9.
- Reset FTN.
- Set the drawing sample rate lower, see Appendix 5.
- If the same error persists, turn off the power of FTN and turn on the power of FTN.
- If the same error persists again, reboot UPLC and PC system, see Appendix3.

Error：System not calibrated. Try to Calibrate

エラー　ACQ-TUV#A16T　System not calibrated. Try to Calibrate.

システムはキャリブレーションされていません。キャリブレーションしてください。

メッセージの説明	溶液
システムがキャリブレートされていません、キャリブレーションしてください	検出器を再キャリブレーションします。

- Reset TUV.
- If the same error persists, reboot UPLC and PC system, see Appendix3.

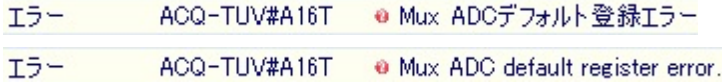
Error：サンプル圧力センサー　ハードウェア不良[Hardware failure of Sample pressure sensor]

サンプル圧力センサーのハードウェア不良

メッセージの説明	溶液
内部的な問題	1. サンプルマネージャーをリセットします。 2. 警告が継続する場合は、電源をいったん切って、再度入れ直します。 3. それでも警告が継続する場合は、Watersテクニカルサービスに連絡してください。

- Reset FTN.
- If the same error persists, turn off the power of FTN and turn on the power of FTN.
- If the same error persists again, reboot UPLC and PC system, see Appendix3.

Error：Mux ADC デフォルト登録エラー[Mux ADC default register error]

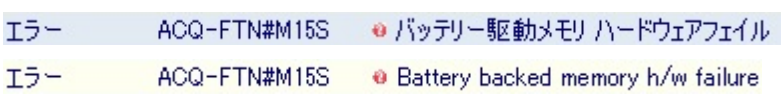


Mux ADC デフォルトレジスターエラー

メッセージの説明	溶液
マルチプレクサ (Mux)アナログデジタルコンバーター(ADC)規定値登録エラー	1. 検出器をリセットします。 2. それでも警告が継続する場合は、検出器の電源を入れ直します。 3. それでも警告が継続する場合は、Watersテクニカルサービスに連絡してください。

- Reset TUV.
- If the same error persists, turn off the power of TUV and turn on the power of TUV.
- If the same error persists again, reboot UPLC and PC system, see Appendix 3.

Error：バッテリー駆動メモリーのハードウェアフェイル[Battery backed memory h/w failure]



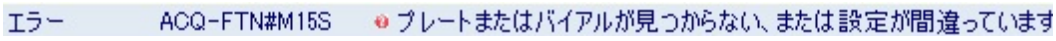
バッテリー駆動メモリーのハードウェアフェイル

注意：すべてのキャリブレーションパラメータが消失した可能性があります。問題の解決後、設定値をリストアするか、キャリブレーションを繰り返す必要があります。

メッセージの説明	溶液
ランプの破損	Watersテクニカルサービスに連絡してください。

- Reboot UPLC and PC system, see Appendix 3.

Error：プレートまたはバイアルが見つからない、または設定が間違っています**[Sample plate or vial is not found, or Incorrect parameters are set.**



プレートまたはバイアルが見つからない、または設定が間違っています

サンプルマネージャーがバイアルまたはプレートの正しい高さを検知しないか、期待したバイアルまたはプレートと異なるものを検知しています。

メッセージの説明	溶液
プレート/バイアルがありません	指定したプレートまたはバイアルが正しい位置にあるか確認します。バイアルが正しい位置にあり、正しいプレートが選択され、ANSIバージョンのプレートが取り付けられていることを確認します。
プレート/バイアルが正しく指定されていません	プレートがソフトウェアで正しく指定されていることを確認します。
プレート/バイアルが期待したものより短い	正しいプレート/バイアルを選択して、ANSIバージョンのプレートを使用します。
内部的な問題	Watersテクニカルサービスに連絡してください。

- Check the sample vial type, vial position and the placement of plate.
- Confirm the vial and plate setting on Empower3, see 3E-6, 7.

Error：ポンプ（ハードウェア）圧力超過**[Overpressure of pump pressure]**

ポンプ(ハードウェア)圧力超過

システム圧力が最大圧力を3450 kPa (34.5 bar、500 psi)上回ると、ポンプハードウェア圧力超過エラーの原因となります。

メッセージの説明	溶液
システムに対して流量が高すぎます。	1. メソッドパラメータを確認し、流量設定が高すぎたり、温度設定が低すぎたりしないか確認します。 2. 送液を停止し、圧力を逃がします。 3. 適切な流量を設定します。(メソッドとシステムで許容されている場合、上限を上げます。)
ベントバルブがブロック位置にあるときに、ユーザーが流量を設定しようとしました。	ベントバルブの交換
ポンプハードウェアの詰まり	ヒント:システムの流路のあらゆる部分が、明らかなベントバルブあるいはカラムの問題の原因となることがあります。 1. ミキサー/フィルタアウトレットのフィッティングを緩め、圧力が低下するかどうかを観察します。低下する場合は、サンプルマネージャーで同じテストを実行します。 2. 圧力が維持されている場合、システムの流路の次のフィッティングを緩め、圧力が低下するかどうかを観察します。 3. ステップ2を繰り返し、圧力の低下が見つかるまで、システムの流路をポンプまで調べます。 4. 問題の原因となっている部分を修理、または交換します。 5. それでも問題が解決しない場合は、Watersテクニカルサービスに連絡してください。
内部的な問題	Watersテクニカルサービスに連絡してください。

- Check the setting values of the flow rate, the system pressure limit and temperature.
- Set the lower flow rate, higher pressure limit or higher temperature.
- Check the clogging of tubing or channel of the system.
  Search the clogging part with loosening the fitting one by one. The direction to search is from the column to the pump. If the clogging parts are identified, clean or change them.

Appendix 2 Solvent Inlet Line

UPLC system has seven solvent inlet line.
A (yellow), B (blue), C (red), D (green): buffer line, [B, C, D (not in use) ->50% Ethanol]
WASH (white): needle wash
SEALWASH (brown): pump seal wash
PURGE (orange): sample syringe purge

Please set solvent inlet lines as described below.
CAUTION: Change the bottles for the inlet line after the flow was stopped.

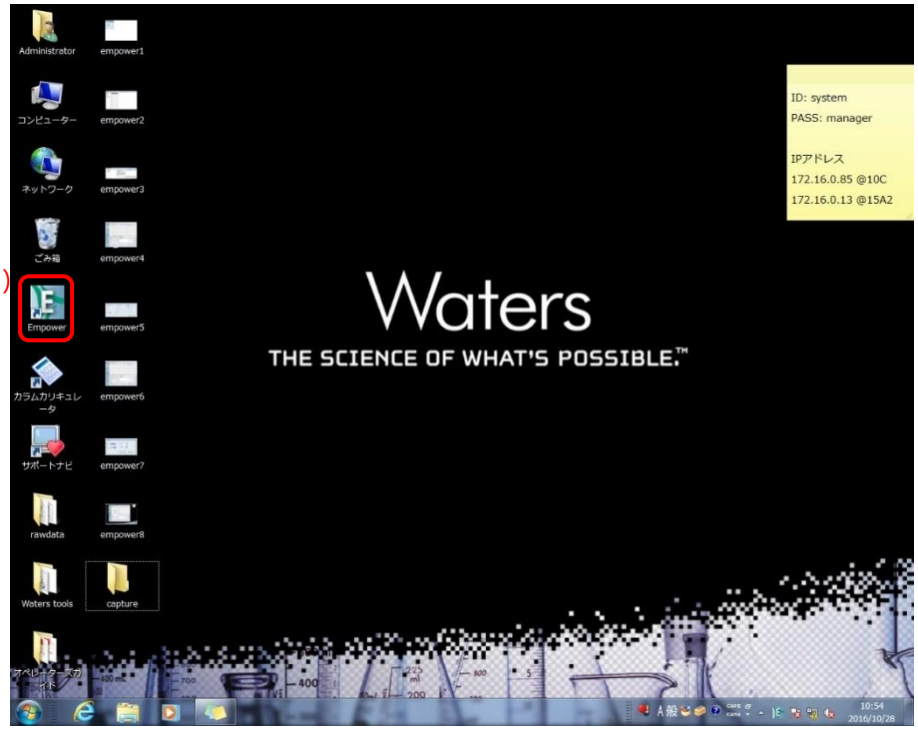
（1）During storage
20% Ethanol：A(yellow), WASH(white), SEALWASH(brown), PURGE(orange)
（2）During flow MilliQ
Milli Q Water：A(yellow), WASH(white), SEALWASH(brown), PURGE(orange)
（3）During flow buffer
Milli Q Water：WASH(white), SEALWASH(brown) , PURGE(orange)
Buffer：A(yellow)

	A(yellow)	WASH(white)	SEALWASH (brown)	PURGE (orange)	B, C, D
Storage	20% Ethanol	20% Ethanol	20% Ethanol	20% Ethanol	50% Ethanol
MilliQ	Milli Q	Milli Q	Milli Q	Milli Q	50% Ethanol
Buffer	buffer	Milli Q	Milli Q	Milli Q	50% Ethanol

Appendix 3 How to start UPLC Control Software (Empower3).

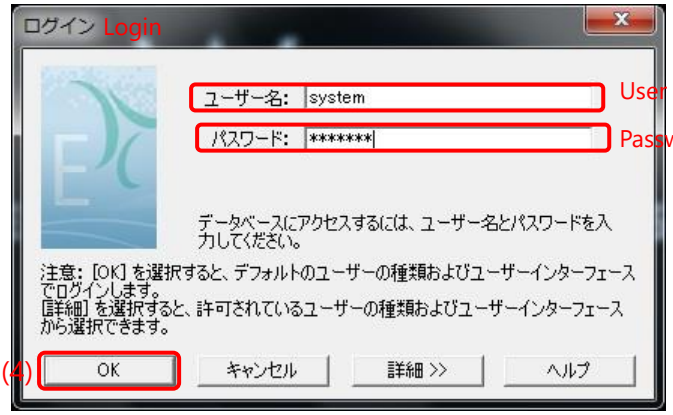
1

Click the Empower3 icon (1) on remote desk top.



2

In the login window, enter user name: system (2), and password: manager (3). And click OK (4).

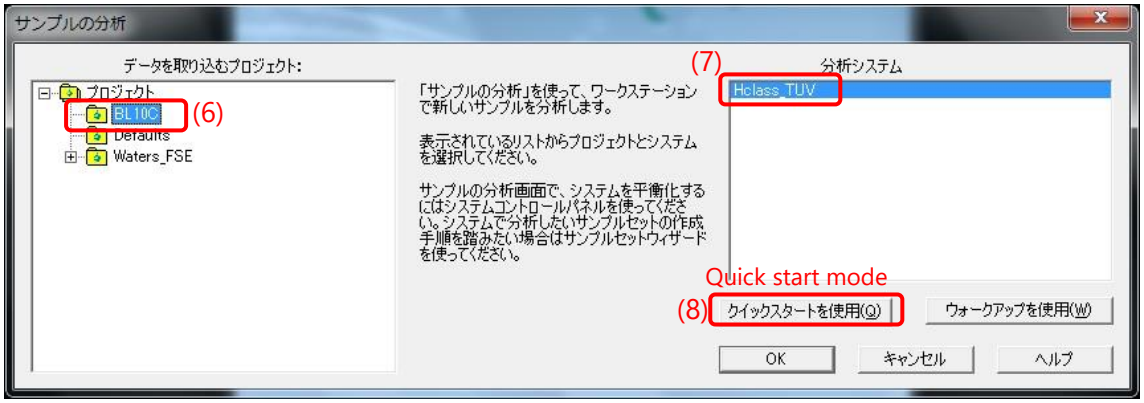


3

Click “サンプルの分析”: Analyze Sample (5) in Empower3 Server Window.

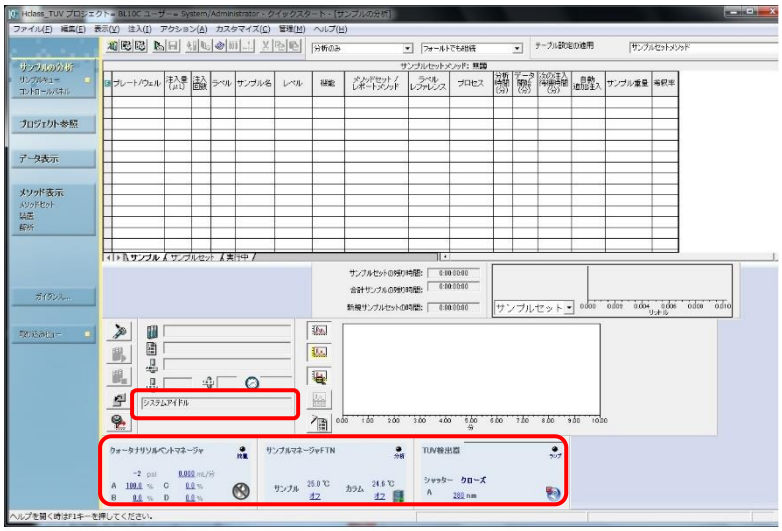
4

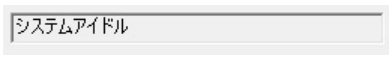
In Analyze Sample Window, select BL10C in [データを取り込むプロジェクト]: Project area (left side) (6), and select [HClass_TUV] in [分析システム]: System area (right side) (7). And then click [クイックスタートを使用]: quick start mode (8).



5

Confirm that [システムアイドル]: system idle is displayed in the message area and QSM, FTN and TUV panels are displayed in the direct control panel of Empower3 window.





System idle -> initializing OK

6

When any error message is appeared in the message area. Perform the re-recognition of equipment.

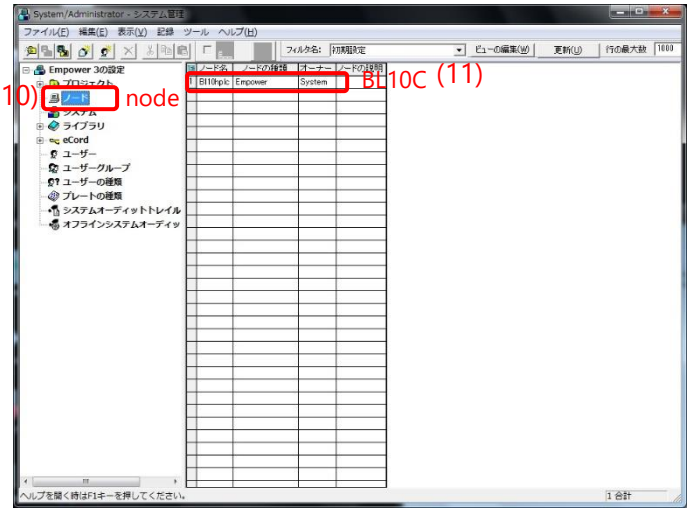
Close the quick start window, and click [システムの再構成]: reconfiguration of system (9) in Empower3 Server Window.

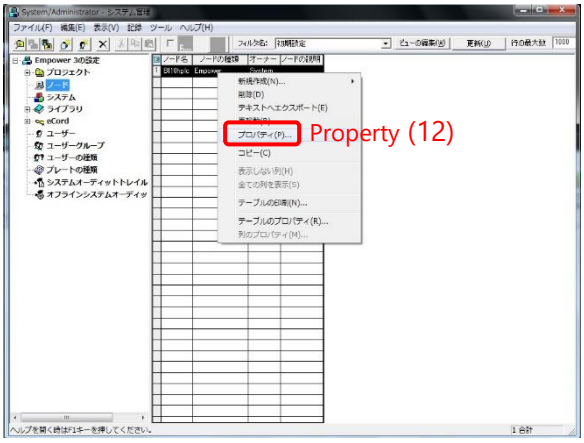
(9) Reconfiguration of system

7

Select [ノード]: node (10) in system management window, and right-click [BL10C] (11) and

select [プロパティ]: property (fifth line) (12) in the center table.





8

Confirm that [OK?] column in equipment tab of property window is [yes]. If three columns are [Yes], three equipment are connected to PC. If more than one column is [No], Click [装置のスキャン]: re-scan (13) to retry to connect to PC.

9

Click [OK] (14) in property window and close the system management window, and return to Step3.

10

If re-start steps fail, Close all Empower3 windows and return to Step1.

If re-re-start steps fail, reboot PC and UPLC sytem.

Shut down procedure

Check that UPLC flow stop.

Close Empower3 windows.

Shut down PC

Turn off UPLC (QSM, FTN, TUV (front side) and Column cooler (right side)).

Reboot system

Turn on Column cooler.

Turn on PC and wait until Windows OS start.

Turn on QSM, FTN and TUV and wait until initializing equipment (several minutes).

Start Empower3.

Start remote desktop from the control PC outside the experimental hatch.

The short cut icon of the remote desktop is placed on the desktop of the control PC.

- BL-10 用 HPLC@10C
- HPLC リモート@15A2

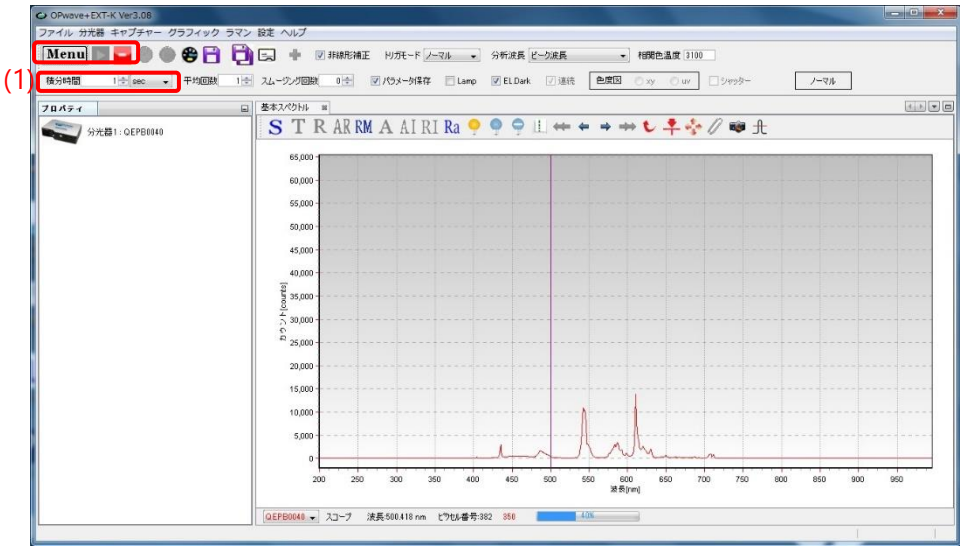
Appendix 4 How to start UV spectrophotometer control software [OPwave+EXT-K]

1

Click OPwave+EXT-K icon.

2

Confirm that [積分時間]: integration period is [1sec] in OPwave+EXT-K (UV spectrophotometer software). If not, set [1sec] (1).



Integration time

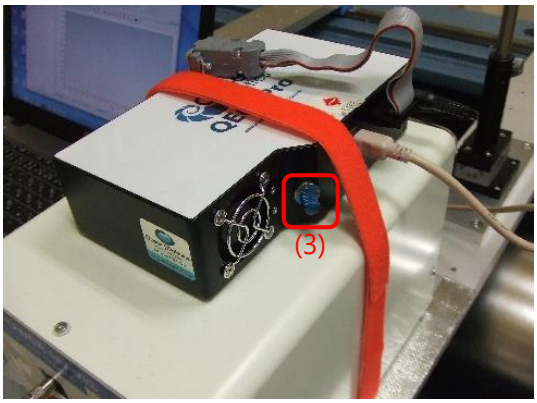
3

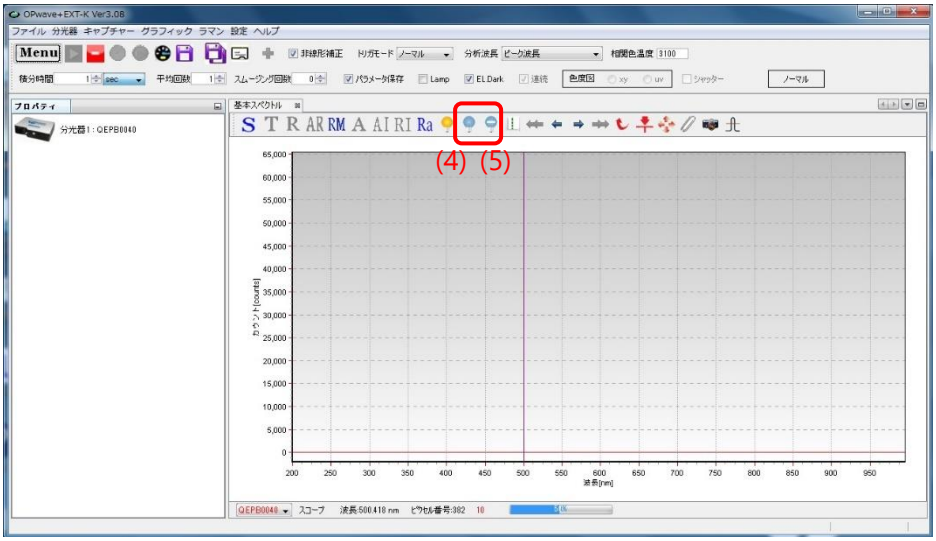
Confirm that OPwave+EXT-K is [live mode]. If the icon next to menu icon is grey and the spectra is renewed every sec, OPwave+EXT-K is set to [live mode]. If the icon next to menu icon is green, Click that green icon (2), and set OPwave+EXT-K to [live mode].

Live mode

4

Loosen and remove light fiber cable from UV spectrophotometer, and install metal cap to shield from light (3). Click dark save icon (blue bulb) and click minus dark icon (blue bulb with white bar). The icon will be changed to blue bulb with red bar.





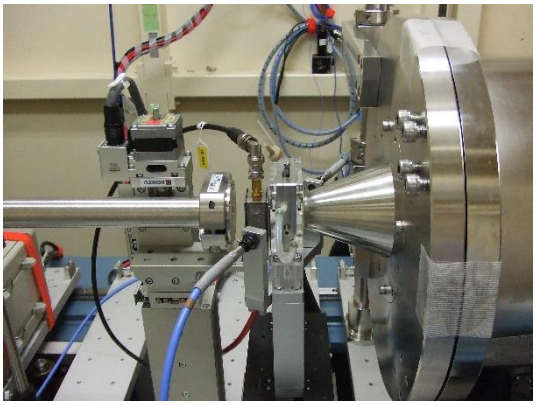
dark subtracted

5

Re-connect UV spectrophotometer and light fiber cable. Confirm that the shutter of light source is open, if not, push shutter button of light source (6).

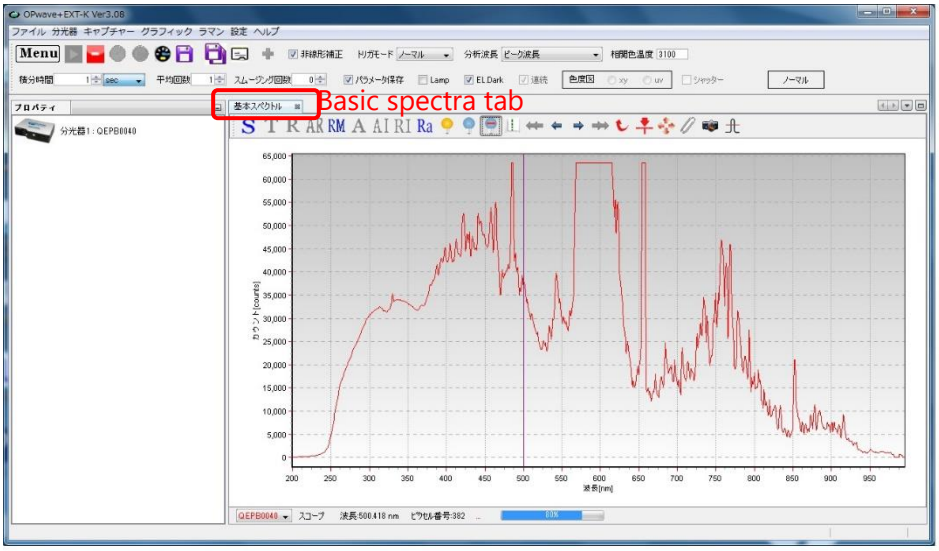
6

Set SAXS flow cell (filled up with MilliQ or buffer) to cell holder.



7

Select [基本スペクトル]: Basic spectra tab in OPwave+EXT-K.



8

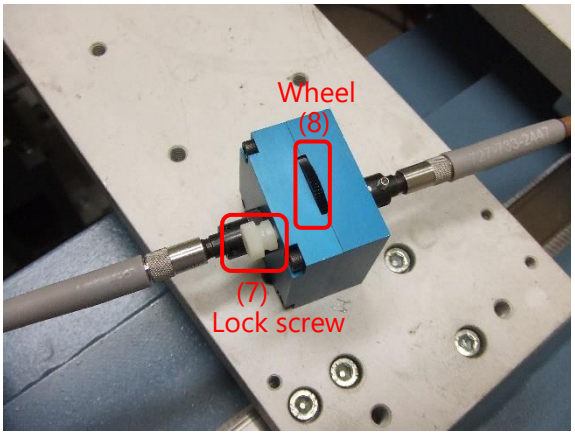
Adjust light intensity with attenuator (actual maximum counts should be less than 70~80 % of maximum (QE65Pro: 16bit, QEpro: 18bit).

(Example: when transparent protein solution is measured (range 220 – 400 nm), adjust about 30000 counts (QE65Pro) and 120000 counts (QEpro) [about 70% of maximum]).

How to adjust with attenuator

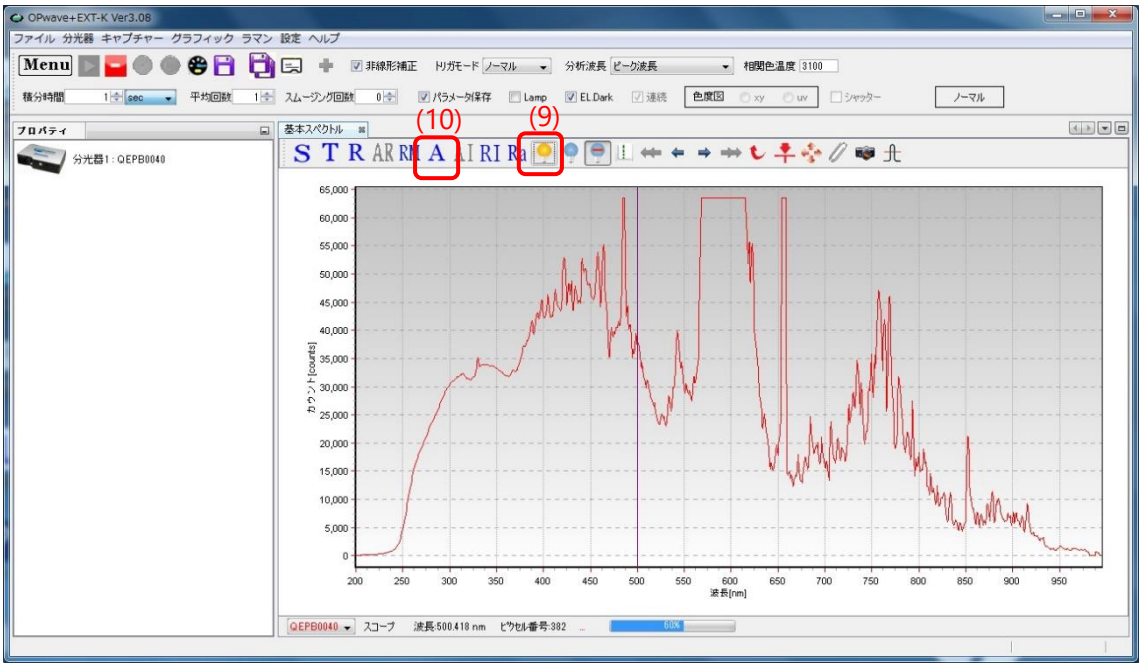
Loosen white lock screw (7) and rotate black wheel (8) to adjust aperture.

(When the lock screw will be tightened, light intensity is easy to change.)



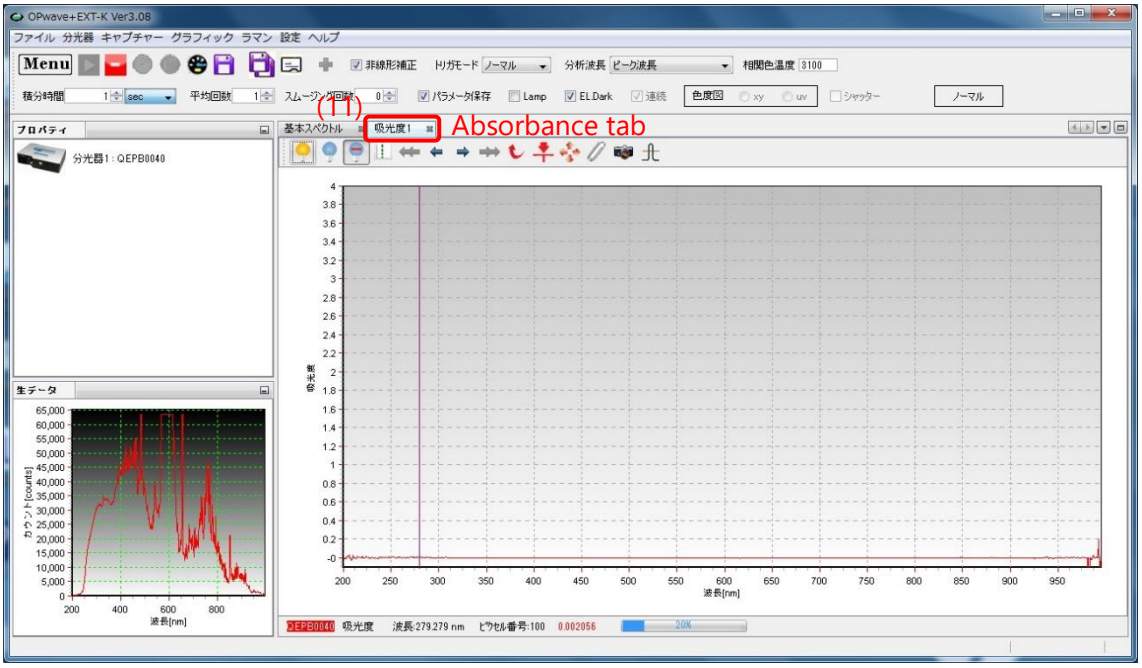
9

Click baseline icon (yellow bulb) (9) to make reference, and then click [A] icon (Absorbance mode) (10) to make absorbance tab. Select [吸光度 1]: absorbance 1 tab (11).



baseline (9)

A(Absorbance mode) (10)



10

Click the remote desktop icon of the measurement PC outside experimental hatch.

- Flow&QE65pro Remote Desktop@10C
- UV for HPLC & Solution Flow@15A2

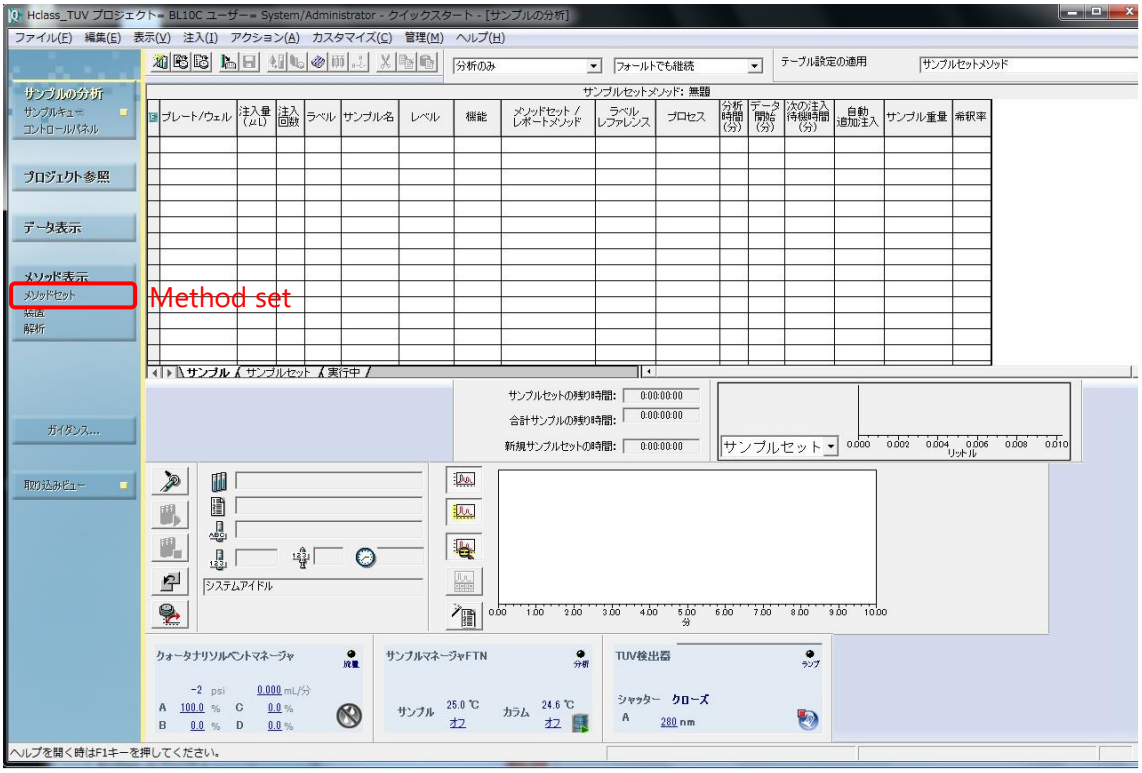
Appendix5 How to change the drawing sample rate

If errors about the sample injection occur frequently, Try to set the drawing sample rate lower.

Warning：サンプル吸引率の超過[aspirated sample volume is low.], see Appendix 1.
Error：サンプル送液最大圧力[Sample fluidics high pressure limit.] , see Appendix 1.

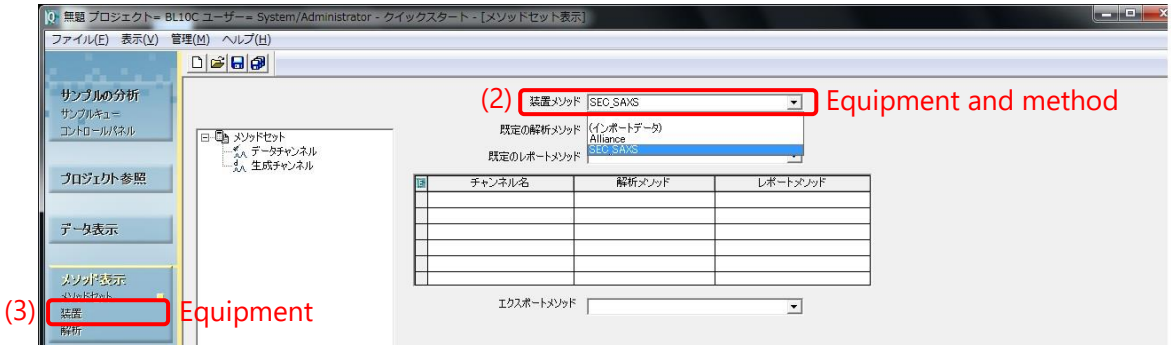
1-1

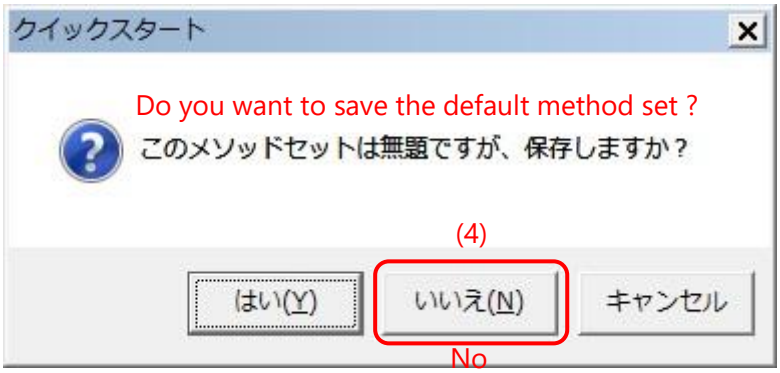
Click [メソッドセット]: Method set (1) in the navigation bar (left side of UPLC control software).



Select [SEC-SAXS] (2) in the [装置メソッド]: equipments and method, and click [装置]: equipments (3) in the navigation bar.

[クイックスタート]: quick start window will open, select [いいえ]: No (4).



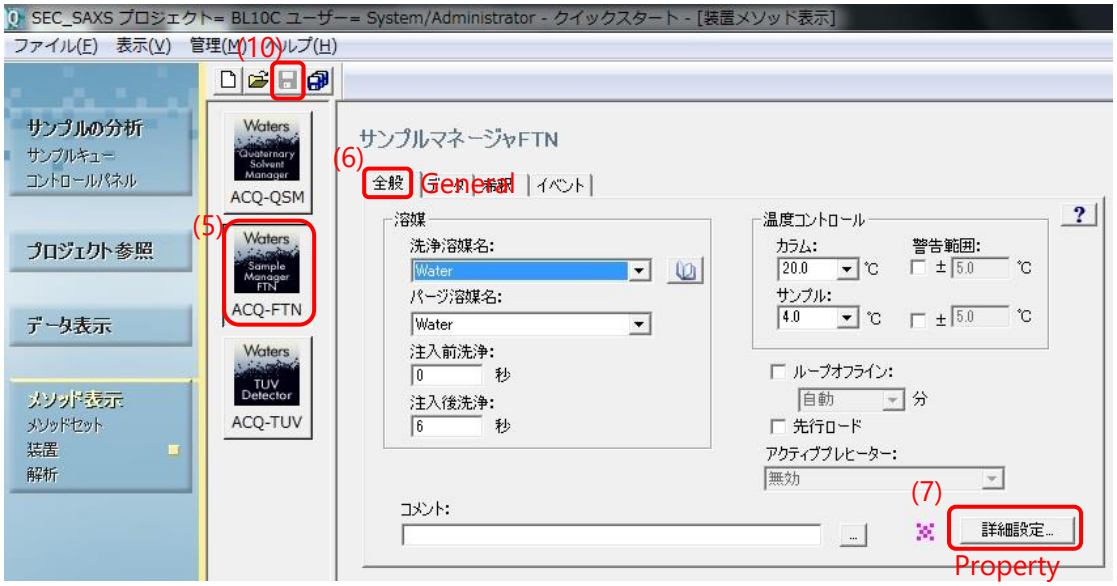


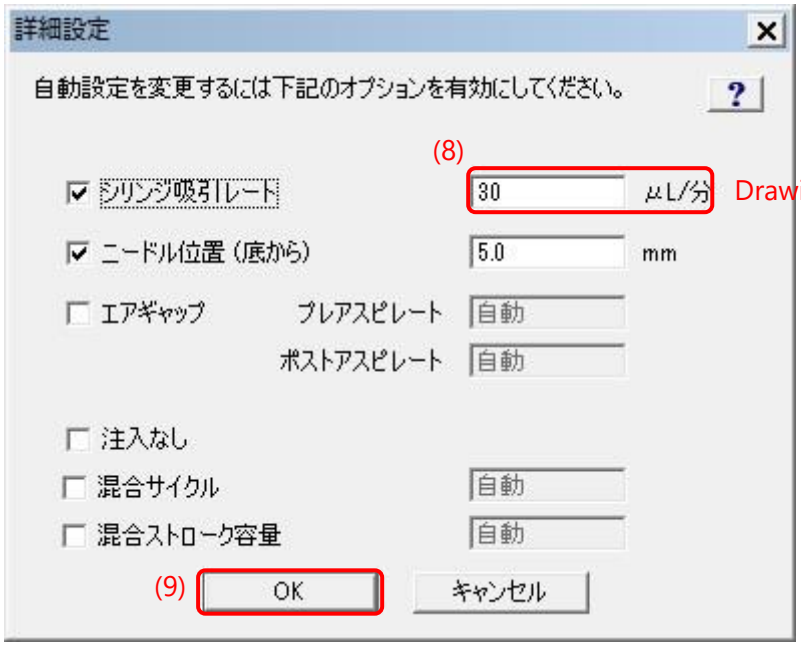
1-2　Setting the drawing sample rate

Click [ACQ-QSM] icon (5), and move to [全般]: general tab (6).

Click [詳細設定]:property icon(7), and set the drawing sample rate in the property window(8). And then click [OK] (9) to close the property window.

（The drawing sample rate has been set to 30ul/min from July 2018. If errors about the sample injection occur frequently, try to set the drawing sample rate 15ul/min.）

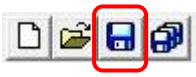




1-2

Click save icon (10) to save modified method.

※To save parameters is needed to run modified method. If you do not click save icon, UPLC will not work proper method.



1-3

After your beam time, restore the drawing sample rate and save modified method again.