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1.0 Purpose

This is a general procedure for operation of BIAcore 3000 system to determine the affinity of a protein / antigen (analyte) to its antibody or receptor (ligand) or other ligand / ligand interactions using BIAcore.

2.0 Scope

This SOP is to be used for the quantitative determination of binding of an analyte to its ligand.

3.0 Authority and Responsibility

3.1 The Director, Technical Operations, Process Analytics\Quality Control (PA\QC) has the authority to define this procedure.

3.2 The Manager or designee, PA\QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA\QC personnel are responsible for the performance of this procedure.

3.4 PA\QC personnel are responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Equipment and Materials

4.1 BIAcore 3000 (MEF #71360; Serial No. 33-17339-3360.

4.2 Sartorius Water System (MEF #LWPS-015-B).

4.3 Sensor chip CM5 –Research Grade (BDP PN 22062) or alternate chips for specific use.

4.4 Sensor chip CM5 –Certified Grade (BDP PN 22063) or alternate chips for specific use.

4.5 7 mm polypropylene vials (BDP PN 22064 or BDP approved equivalent).

4.6 Caps for 7 mm polypropylene vials (BDP PN 22065 or BDP approved equivalent).

4.7 9 mm glass vials (BDP PN 22076 or BDP approved equivalent).

4.8 16 mm glass vials (BDP PN 22066 or BDP approved equivalent).

4.9 Caps and Septa for 16 mm glass vials (BDP PN 22067 or BDP approved equivalent).

4.10 10 µL (Rainin L-10 or equivalent), 200 µL (Rainin L-200 or equivalent) and 1 mL (Rainin L-1000 or equivalent) Pipetman.

4.11 10 µL (BDP PN 21472 or BDP approved equivalent), 200 µL (BDP PN 21470 or BDP approved equivalent), and 1 mL (BDP PN 21471 or BDP approved equivalent), filter pipet tips.

4.12 1.5 mL Eppendorf tubes (BDP PN 20659 or BDP approved equivalent).

5.0 Reagents

5.1 Amine coupling kit (BDP PN 30969), (contains NHS, EDC and 1M Ethanolamine).

5.2 BIAcore maintenance kit (BDP PN 30971), (Contains a system maintenance chip and testing and cleaning solutions).

NOTE: The reagents specified are for amine coupling. When an alternate coupling procedure is to be used, reagents specific for the coupling mechanism may be used and documented).

6.0 Buffers and Solutions

6.1 HBS-EP Buffer - 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20 (BDP PN 30967).

6.2 HBS-N Buffer - 0.01 M HEPES pH 7.4, 0.15 M NaCl (BDP PN 30968).

6.3 10 mM Sodium Acetate, pH 5.5 (BDP PN 30970).

6.4 10% SDS (BDP PN 30532 or BDP approved equivalent).

NOTE: Buffers and solutions may be varied depending on the specific use. Document the product specific details in specific procedures.

7.0 Control Reagents and Materials (Project Specific):

7.1 Analyte or reagents are product specific. Document the reagent details in product specific SOPs.

8.0 Procedure

8.1 System Setup

NOTE: For detailed information see Attachment 1.

8.1.1 Turn ON the computer connected to the BIAcore 3000. Create a folder in an appropriate subdirectory and folders in the local disk (C:) that can later be stored in the network under scidata on 'fr-s-bdp-vlan in S:\PA\BioAnalytical Instruments\BIAcore. Use the date that the sensor chip is prepared as the name of this working directory and the format for naming this directory is "mmddyy" (e.g., 111710) and this directory is then known as the working directory.

8.1.2 Turn ON the BIAcore 3000 (Power button is located on the left panel of the machine) and the computer.

8.1.3 Start the BIACORE 3000 CONTROL SOFTWARE. Once the machine is connected to the computer (the lower right side of the Control software window displays this information), following operations are performed in sequence.

8.1.4 The buffer inlet tubes (Fig. 1) are placed in a 200 mL bottle containing distilled, de-ionized, and 0.2µ filtered water or the running buffer.

8.1.5 Docking of a maintenance chip for cleaning (performed once a week if the instrument is being used continuously or before starting a new project). For details see Attachment 1.

8.2 General Sensor Chip preparation procedure: Refer to Attachments 2, 3, and 4 for examples.

NOTE: Sensor chip preparation is product specific and a separate attachment needs to be prepared for each product.

8.3 Surface Performance Test

NOTE: Surface Performance Test is product specific and a separate attachment needs to be prepared for each product.

8.4 Kinetics - Determination of equilibrium dissociation constant (K_D) – See Attachment 5 for an example.

NOTE: Determination of K_D is product specific and a separate attachment needs to be prepared for each product.

8.5 Data analysis - See Attachment 6 for an example.

9.0 Assay Acceptance

9.1 Assay acceptance criteria is project specific and is to be specified on the corresponding form in the project specific SOP (e.g., Form 16139-01.

10.0 Supporting Documents

10.1 BIAcore 3000 Handbook

(S:\PA\BioAnalytical_Instruments\BIAcore\Biacore 3000Instrument Handbookweb.pdf

ATTACHMENT 1

System Set up

1.1 Turn ON the computer connected to the BIAcore 3000. Create a folder in the GMP subdirectory of the C (main) directory. Use the date that the sensor chip is prepared as the name of this working directory and the format for naming this directory is “mmddyy” (eg. 111710) and this directory will be known as the working directory.

1.2 Turn ON the BIAcore 3000 (Power button is located on the left panel of the machine) and the computer.

1.3 Start the BIACORE 3000 CONTROL SOFTWARE. Once the machine is connected to the computer (the lower right side of the Control software window displays this information), following operations are performed in sequence.

Fig 1. BIAcore 3000 (Front view)

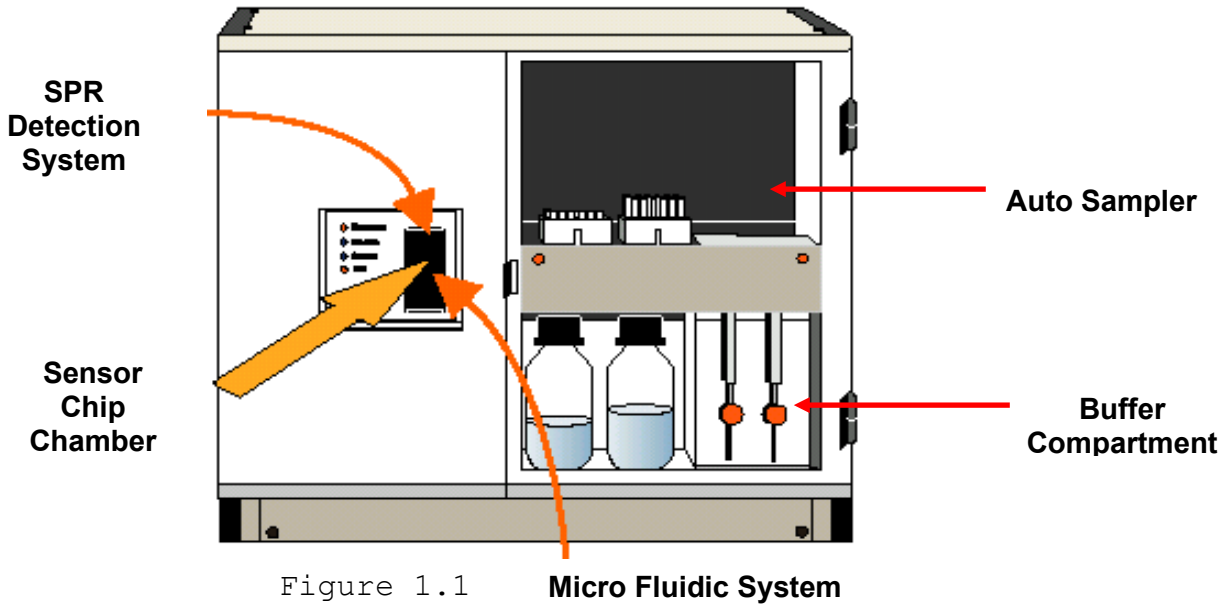


Figure 1.1

ATTACHMENT 1 (Continued)

1.4 The buffer inlet tubings (Fig. 1) are placed in a 200 mL bottle containing distilled, de-ionized and 0.2 µm filtered water.

1.5 Docking of a maintenance chip:

1.5.1 In the control software window click on

Command

↓

Undock

1.5.2 Open the Sensor chip compartment on the machine (Front Panel - middle left Fig. 1) and remove any previously installed Sensor chip and slide in the maintenance chip.

↓

Dock

1.5.3 Once the sensor chip is docked, the program prompts for “Run PRIME”.

Click Yes**.**

1.5.4 After the priming, perform a DESORB using BIAdesorb solution 1 and BIAdesorb Solution 2 from the BIAmaintenance kit (BIAcore Cat No. BR-1006-66):

1.5.5 In the control software window click on:

Tools

↓

ATTACHMENT 1 (Continued)

Working Tools

↓

Desorb

1.5.6 Click on 'Start'

1.5.7 Click on 'Continue'

1.5.8 The program will prompt to place BIAdesorb solution 1 in Position R2 F3 and BIAdesorb solution 2 in R2 F4 (See Attachment 7 for Rack Positions).

1.5.9 Click on 'Start'

1.5.10 Approximately 15 minutes is required for DESORB to finish.

1.5.11 After DESORB undock the maintenance chip and Dock a System chip (Section 1.5 of Attachment 1 of SOP 16138).

NOTE: The following procedure is used only when performing a release test.

1.5.12 Perform the System Check using BIATest solution 15 % (w/w) sucrose in HBS-EP buffer and a new CM5 Chip. Follow the program prompts.

Perform System Check.

Tools

↓

Test Tools ⟶ System Check

ATTACHMENT 1 (Continued)

Follow program prompts

1.5.13 Save the results of the system check in the working directory and name the file as System_Check_date. (Include the information in this file with the QCTR data package).

1.5.14 If the system passes the test (the results of the system check will be displayed after the testing is over), go to the next step.

1.5.15 If the system check failed the test, first perform 'Unclogging' under Service Tools. This can clear partially blocked IFC flow cells and loops by flushing with buffer at a high flow rate and takes about 5 minutes runtime. Repeat System Check after this. If it fails again then inform supervisor and call for Technical Service.

1.5.16 If the system check passes the test then go to 'Run' and select Run Sensorgram and let the system run after placing the inlet tubings in the appropriate running buffer and let the baseline equilibrate for a few hours or overnight before actual product runs..

NOTE: The BIAmaintenance Kit does not come with a System Check chip. The System Check is performed with a CM5 Chip appropriate for the assay and subsequently used for the assay.

ATTACHMENT 2

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

NOTE: The type of reagents and the volumes mentioned in this attachment are examples. Instead of capturing the data for sensor chip preparation in different sensorgrams and files described below (Attachments 2, 3, and 4), it can be saved in a single file and all the relevant information is captured on the project specific form which is associated with the project specific SOP (e.g., Form 16139-01).

2.1 Start BIAcore control software.

2.2 From File → Open the template file H:\5PA\PAOnly\\BioAnPublic\Biacore\Templates\ SurfacePrep_activations_flowcell1.

2.3 Write a brief note in the Notebook section. An example is shown in Figure 2.1.

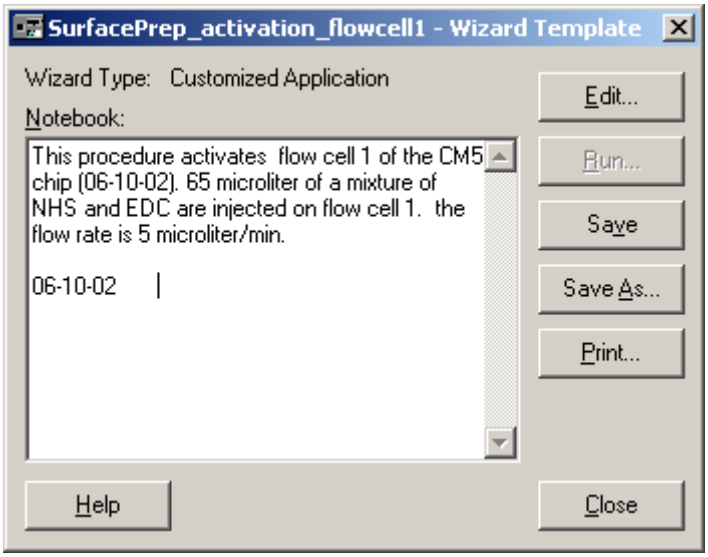


Figure 2.1.

2.4 Click on Edit

2.5 This will open up the sequence window (Fig. 2.2)

ATTACHMENT 2 (Continued)

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

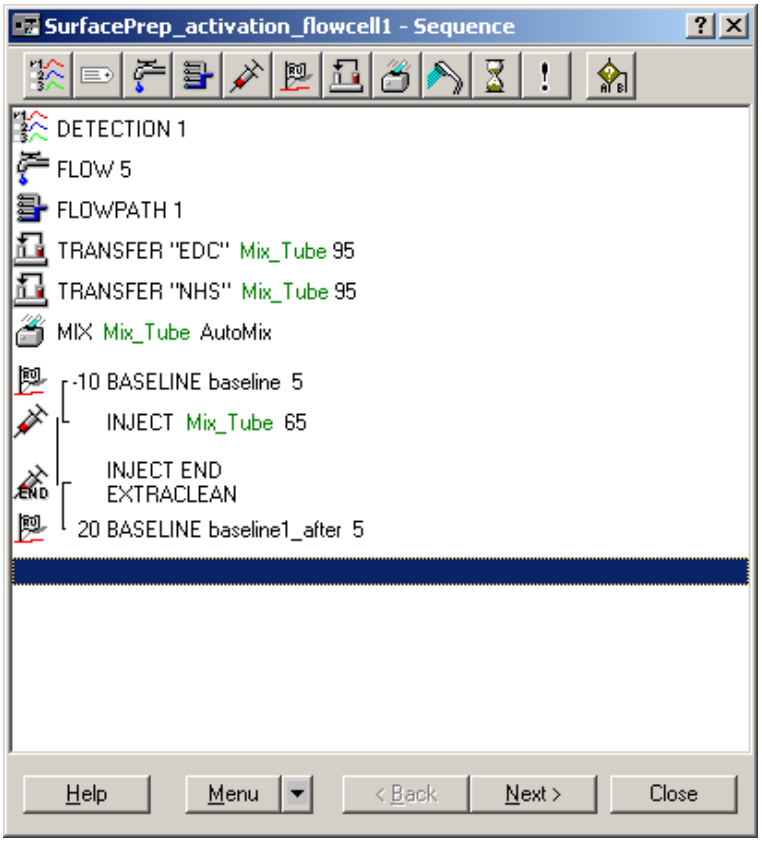


Figure 2.2 Sequence Window

2.6 Step 1

2.6.1 Double click on Detection 1 (1 denotes the flow cell number) - Cycle settings (Fig. 2.3) window will open.

2.6.2 Select Detection Mode.

2.6.3 From the pull down menu of ‘Detection’ Select Fc1 (Flow cell 1).

2.6.4 Click OK. Sequence Window (Fig 2.2) will be displayed again.

ATTACHMENT 2 (Continued)

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

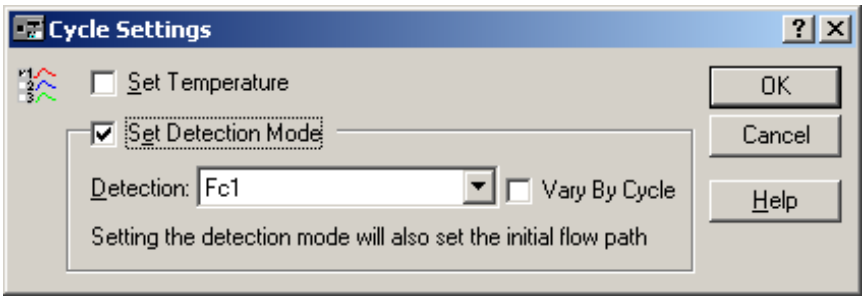


Figure 2.3 Flow cell selections

2.7 Step 2

2.7.1 Double click in Flow

2.7.2 The Flow setting window (Fig. 2.4) will open

2.7.3 Set the flow rate to 5 µL/min

2.7.4 Click OK. Sequence Window (Fig 2.2) will be displayed again

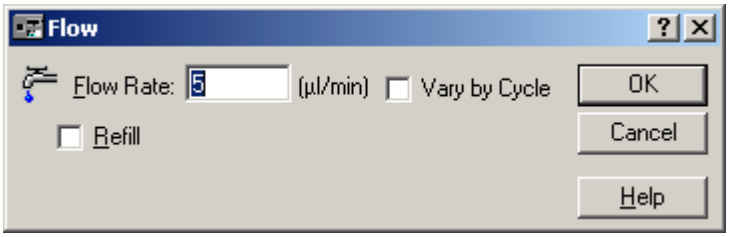


Figure 2.4

2.8 Step 3

2.8.1 Double click on Flowpath

2.8.2 The Flow Path window (Fig. 2.5) will open

2.8.3 Select a Flow Path using the pull down tab

2.8.4 Click OK. Sequence Window (Fig 2.2) is again displayed

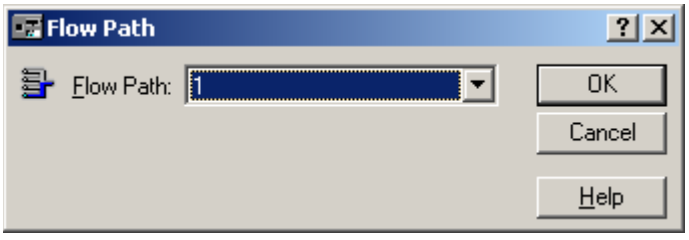


Figure 2.5

ATTACHMENT 2 (Continued)

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

2.9 Step 4 – Transferring 95 µL of EDC to the Empty mixing tube 'Mix_Tube'

2.9.1 Double click on 'Transfer "EDC" Mix_Tube 95'.

2.9.2 Transfer window (Fig. 2.6) will open.

2.9.3 Type in the information as shown in the Transfer window (Fig 2.6).

2.9.4 Click OK. Sequence Window (Fig 2.2) will be displayed.

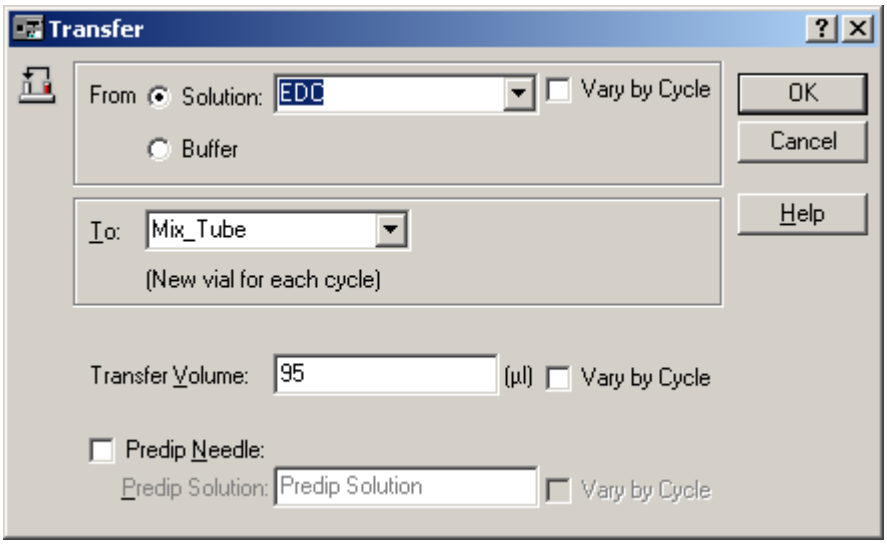


Figure 2.6

2.10 Step 5 - Transferring 95 µL of NHS to the Empty mixing tube 'Mix_Tube'

2.10.1 Double click on 'Transfer "NHS" Mix_Tube 95'.

2.10.2 Transfer window (Fig. 2.7) will open.

2.10.3 Type in the information as shown in the Transfer window (Fig 2.7).

2.10.4 Click OK. Sequence Window (Fig 2.2) is again displayed.

2.11 Step 6- Mixing of NHS and EDC in the Mix_Tube

2.11.1 Double click on 'MIX Mix_Tube AutoMix'.

2.11.2 Transfer window (Fig. 2.8) will open.

2.11.3 Type in the information as shown in the Mix window (Fig 2.8).

2.11.4 Click OK. Sequence Window (Fig 2.2) will be displayed.

ATTACHMENT 2 (Continued)

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

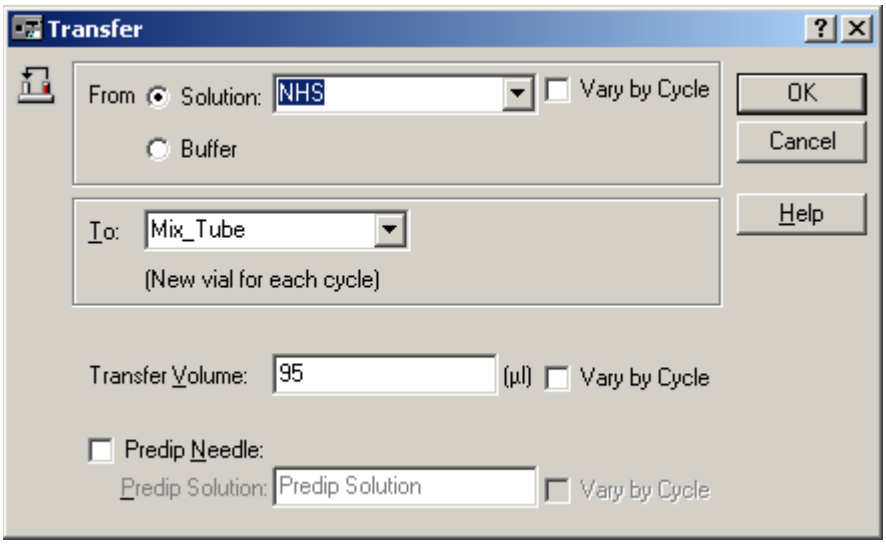


Figure 2.7

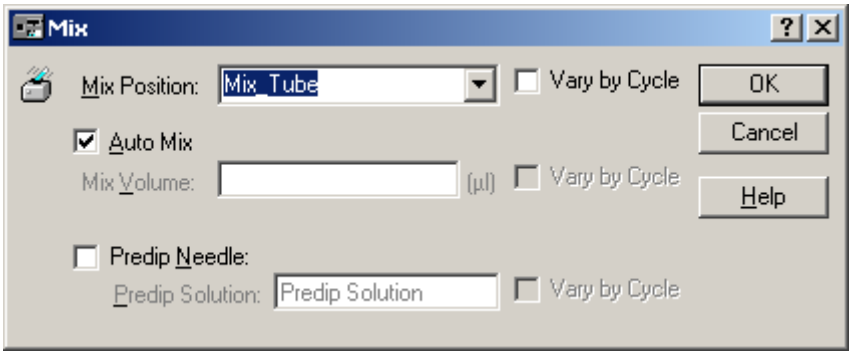


Figure 2.8

2.12 Step 7 – Report Point on the Baseline before injection

2.12.1 Double click on –10 BASELINE ‘Baseline 5’.

2.12.2 Report Point window (Fig. 2.9) will open.

2.12.3 Type in the information as shown in the Report Point window (Fig 2.9).

2.12.4 Click OK. Sequence Window (Fig 2.2) is again displayed.

ATTACHMENT 2 (Continued)

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

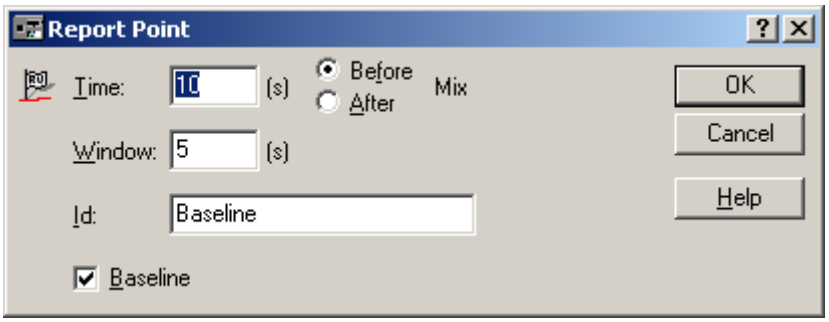


Figure 2.9

2.13 Step 8 – Inject 65 µl of the NHS + EDC mixture from Mix_Tube

2.13.1 Double click on Inject Mix_Tube 65

2.13.2 Inject window (Fig. 2.10) will open

2.13.3 Type in the information as shown in the Inject window (Fig 2.10)

2.13.4 Click OK. Sequence Window (Fig 2.2) will be displayed

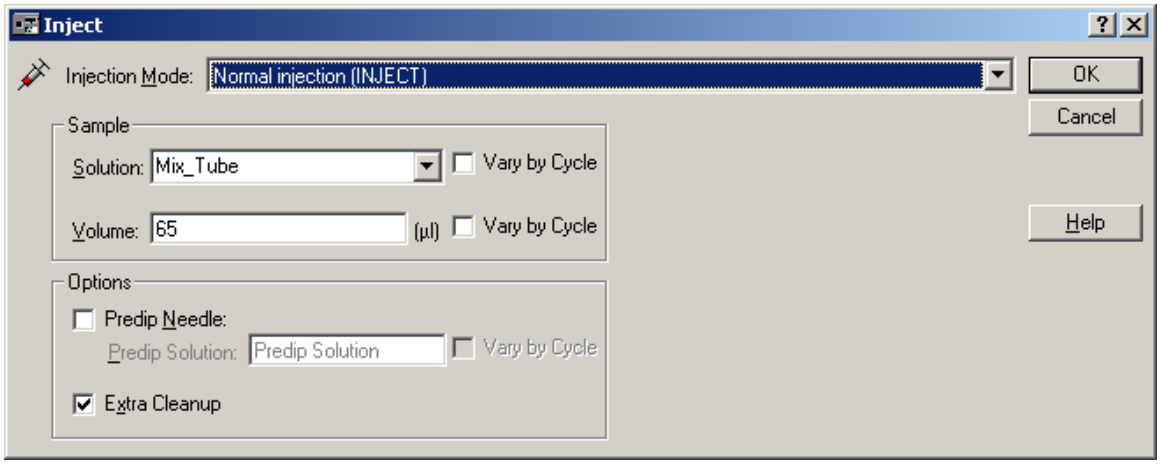


Figure 2.10

2.14 Step 9 – Report Point on the Baseline after injection

2.14.1 Double click on –20 BASELINE ‘Baseline_after 5’.

2.14.2 Report Point window (Fig. 2.11) will open.

2.14.3 Type in the information as shown in the Report Point window (Fig 2.11).

2.14.4 Click OK. Sequence Window (Fig 2.2) will be displayed.

2.14.5 Click on ‘Next’.

ATTACHMENT 2 (Continued)

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

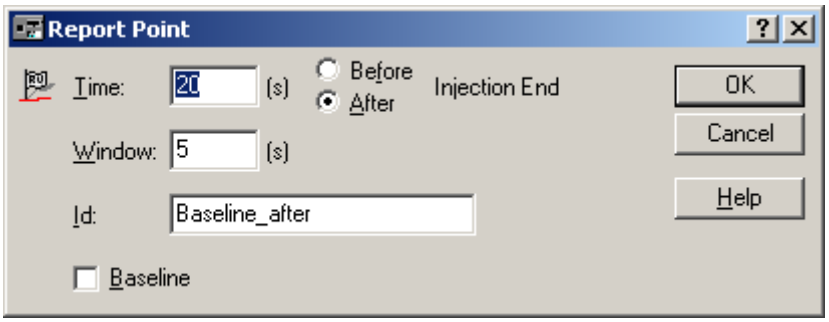


Figure 2.11

2.15 Number of cycles set up

2.15.1 Number of cycles to be run should be set to 1 (Fig 2.12)

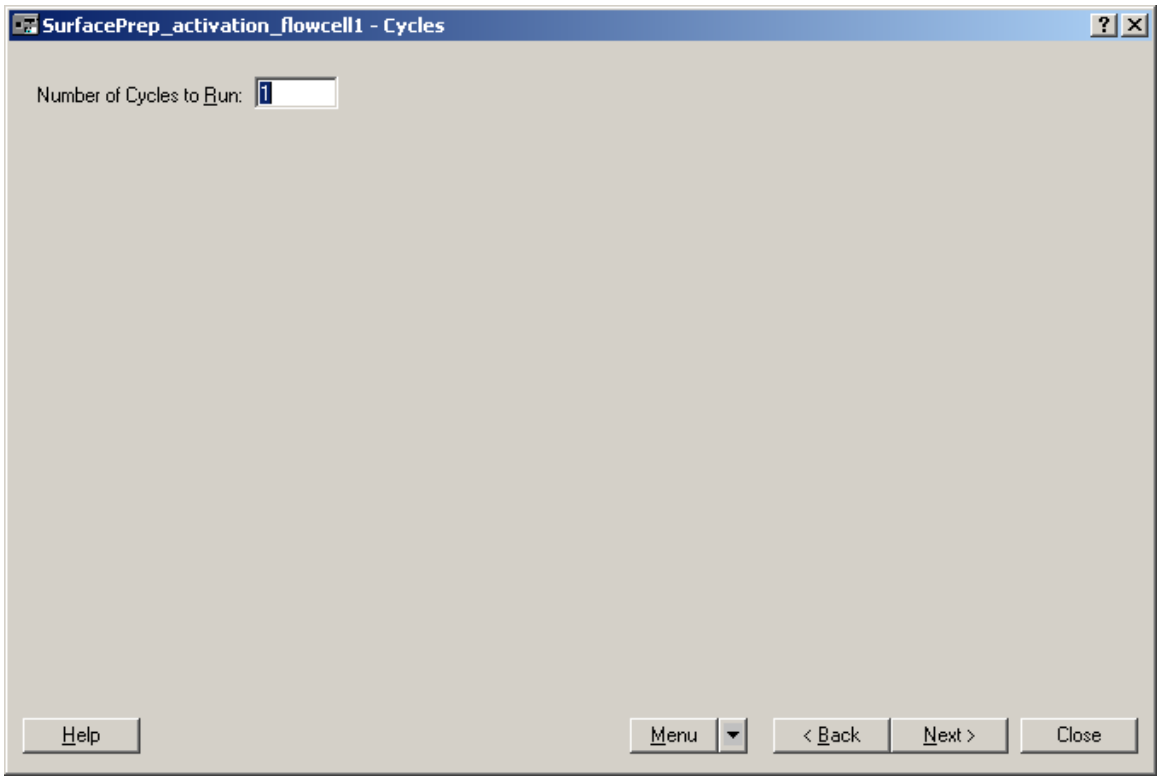


Figure 2.12

2.15.2 Click on 'Next'

ATTACHMENT 2 (Continued)

Procedure for Setup of a Wizard Template for Activation of Flow Cell 1 as an Example

2.16 Rack Positions

2.16.1 Place 110 □L of NHS and EDC into 7 mm polypropylene vials and an empty vial into the rack positions displayed in figure 2.13. (The location of each reagent can be changed by the 'Drag and Drop' method (Hold the left mouse key to drag and drop).

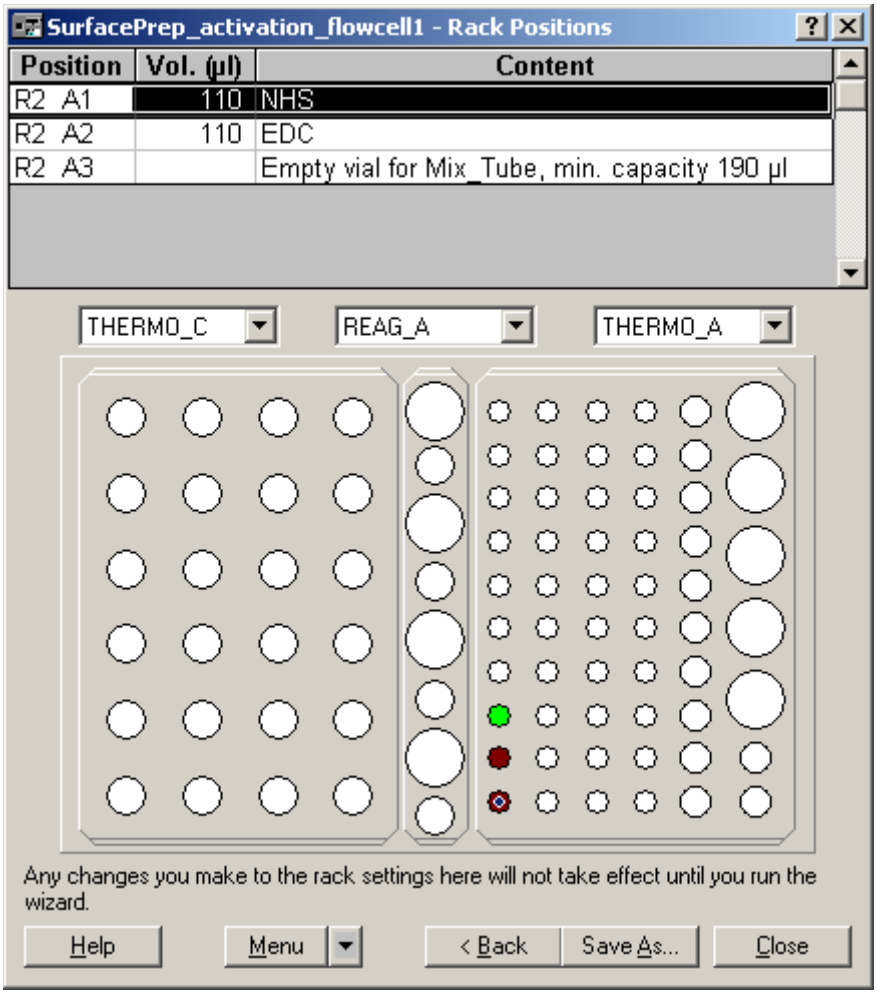


Figure 2.13

2.16.2 Save the method file as 'SurfacePrep_Activation_Flowcell1' in the working directory. (If other flow cells are being used, name the file with the corresponding flow cell number).

ATTACHMENT 2 (Continued)

2.16.3 Run this method file for the activation of flow cell1 using the window shown in figure 2.14. Click on 'Start' for initiating the procedure.

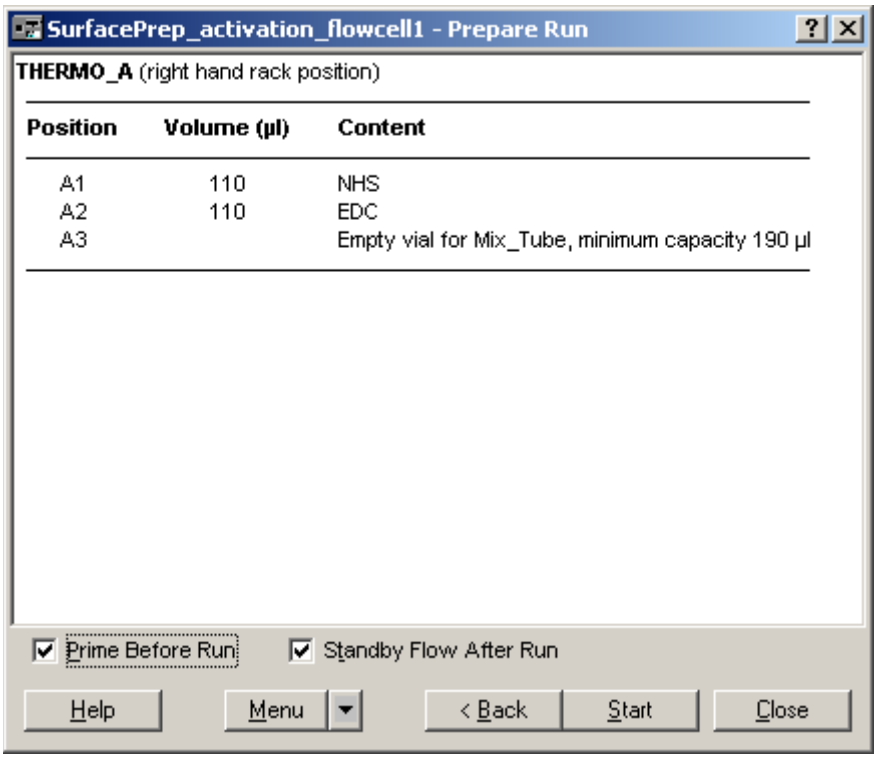


Figure 2.14

NOTE: The activation of the other flow cells in the sensor chip can be performed by setting up the corresponding flow cell numbers in the 'Detection' and 'Flow Path' windows in the same program and save it under a different file name. Activate only the required number of flow cells but a minimum of two – one flow cell as the control surface and the other with the ligand (flow cell 1 and 2 or flow cells 3 and 4 can be used as a pair). In case of using all the four flow cells, flow cell 1 can be used as a control and the other three containing the ligand. Immobilization procedure (next section) must be performed immediately (within 20 minutes) after the activation.

ATTACHMENT 3

Surface Immobilization with a Ligand

NOTE: This procedure describes a general method for immobilizing a ligand to a flow cell. The amount of the ligand and dilution buffer used for the immobilization is product specific and the information will be captured in the form associated with the project specific SOP.

3.1 Start the BIAcore control software.

3.2 From the pull down menu, open 'Run'.

3.3 Choose 'Run Sensorgram'.

3.4 'Detection Mode' window (figure 3.1) will open.

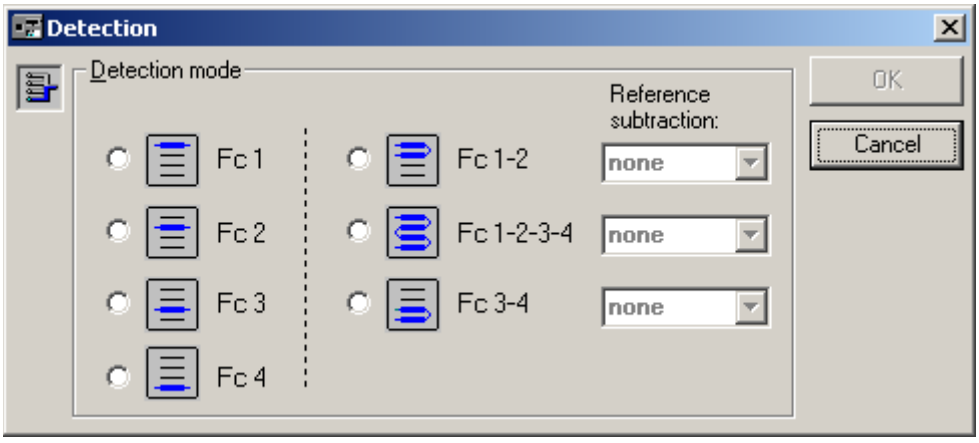


Fig 3.1

3.5 Select the flow cell (e.g., Fc1, Fc2, Fc3, or Fc4).

3.6 Select the appropriate flow path from the 'Command' pull down menu.

3.7 Place the diluted ligand in the autosampler.

3.8 Once the sensorgram is running, an 'Inject Sequence' window will open.

3.9 From the pull down menu, open 'Command'.

3.10 Select 'Inject'.

ATTACHMENT 3 (Continued)

3.10.1 Enter the volume of the diluted ligand to be injected.

3.10.2 Enter the place value of the spot in the autosampler where the diluted ligand is located.

3.11 After the injection is complete, determine the absolute RU after the immobilization (see below).

Absolute RU Determination

3.11.1 Open an existing sensorgram file using the BIAcore 3000 Control software. (This procedure can also be performed on a sensorgram that is running).

3.11.2 Click on 'View'

3.11.3 Click on 'Reference Line' for displaying a cross wire.

3.11.4 Using the left mouse, drag the vertical line of the cross wire to the baseline before the start of the injection. (The horizontal line should overlap with the part of the baseline that is of interest).

3.11.5 Press once on 'F9' key. This will zero the RU of the sensorgram at this point and the value will be displayed in the lower right corner window of the sensorgram. (Pressing 'F9' key again will result in the display of the absolute RU value).

3.11.6 Drag the vertical line to the part of the sensorgram after the injection. Read off the difference RU directly from the lower right corner window.

3.11.7 Click on Reference Line' again to remove the cross wires.

3.12 Continue injections until the required absolute RU is attained.

3.13 Select 'Stop Sensorgram' once the required immobilization is attained.

3.14 Save the sensorgram in the appropriate folder (eg. "SurfacePreparation_Flowcell- x _Immobilization").

ATTACHMENT 4

Surface Deactivation

NOTE: This procedure is performed after the activation (in control flow cell) and immobilization of the ligand.

4.1 Start BIAcore control software.

4.2 From File → Open the template file H:\5PA\PAOnly\Bioanalytical\BioAnPublic\Biacore\Templates\SurfacePrep_Deactivation_Flowcell1 (Wizard Template window will open).

4.3 Write a note in the Notebook part similar to that shown in Figure 4.1.

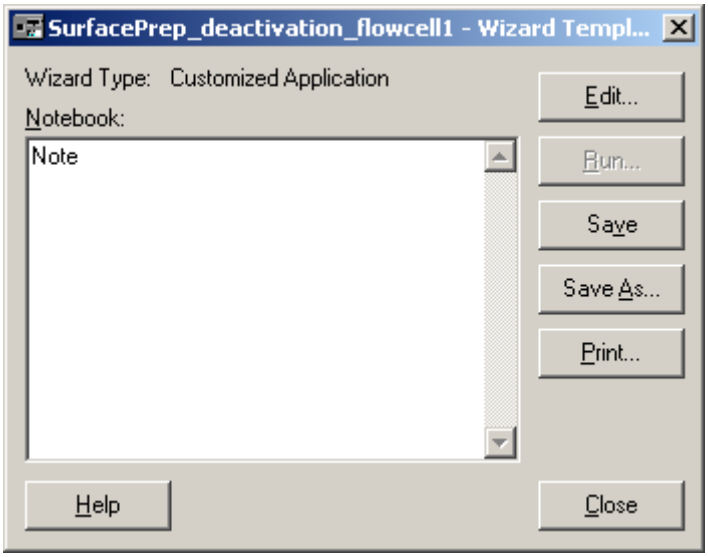


Figure 4.1

4.4 Click on Edit

4.4.1 This will open up the sequence window (Fig. 4.2).

ATTACHMENT 4 (Continued)

Surface Deactivation

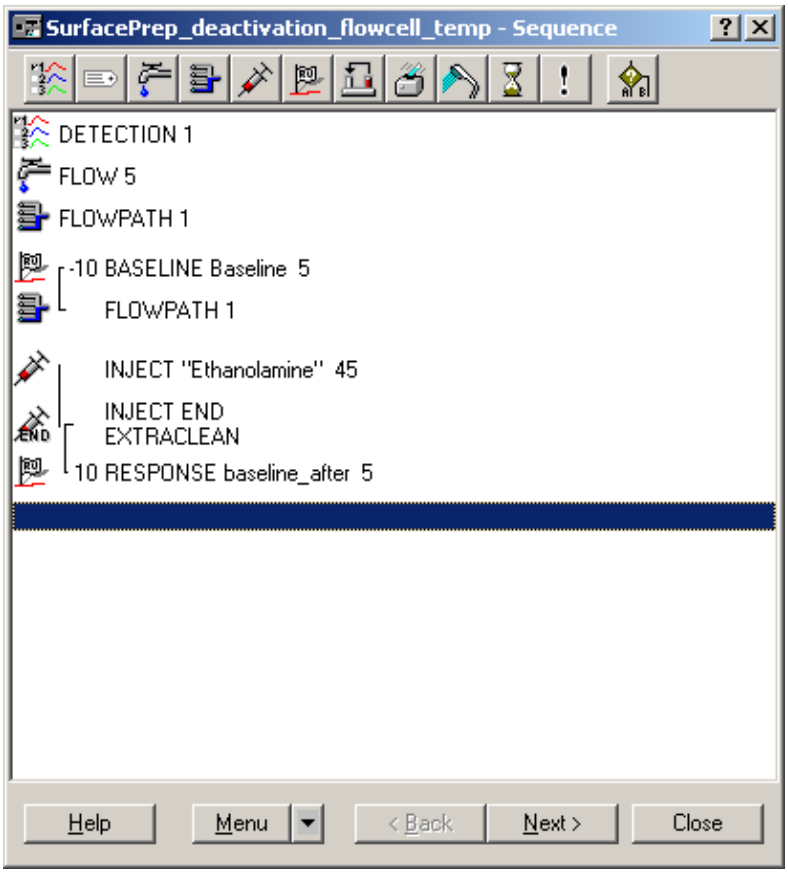


Figure 4.2

4.4.2 Step 1

4.4.2.1 Double click on ‘Detection 1’ (1 denotes the flow cell number) -Cycle settings (Fig. 4.3) window will open.

4.4.2.2 Select Detection Mode.

4.4.2.3 From the pull down menu of ‘Detection’ Select Fc1 (Flow cell 1).

4.4.2.4 Click OK.

4.4.2.5 Sequence Window (Fig 4.2) will be displayed.

ATTACHMENT 4 (Continued)

Surface Deactivation

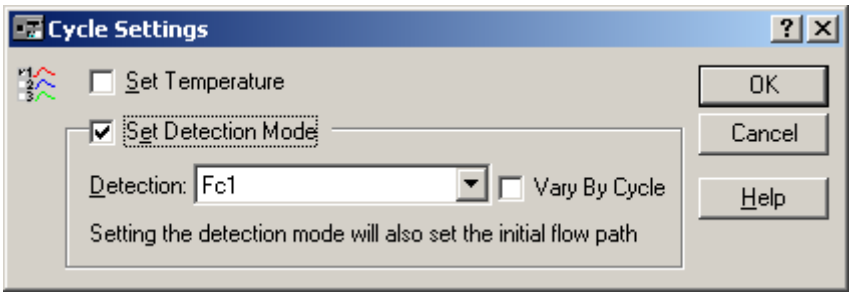


Figure 4.3 Flow cell selection.

4.4.3 Step 2

4.4.2.1 Double click on ‘Flow’.

4.4.2.2 Flow setting window (Fig. 4.4) pops up.

4.4.2.3 Set the flow rate to 5 µL/min.

4.4.2.4 Click OK.

4.4.2.5 Sequence Window (Fig 4.2) will be displayed.

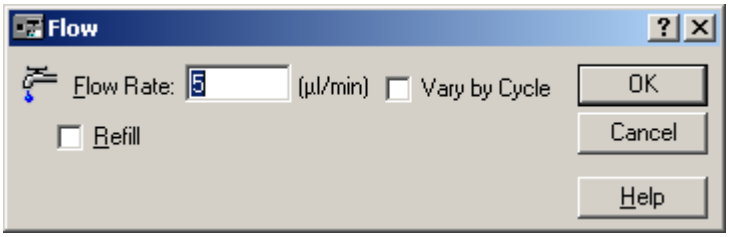


Figure 4.4

4.4.4 Step 3

4.4.3.1 Double click on ‘Flowpath 1’

4.4.3.2 Flow Path window (Fig. 4.5) will open.

4.4.3.3 Select the Flow Path using the pull down tab

4.4.3.4 Click OK.

4.4.3.5 Sequence Window (Fig 4.2) will be displayed

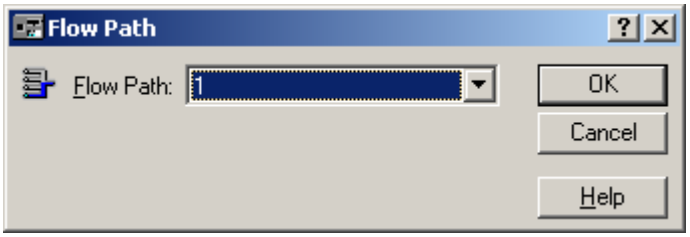


Figure 4.5

ATTACHMENT 4 (Continued)

4.4.5 Step 4 – Report Point on the Baseline before injection

4.4.4.1 Double click on –'10 Baseline baseline 5'

4.4.4.2 Report Point window (Fig. 4.6) will open.

4.4.4.3 Type in the information as shown in the Report Point window (Fig. 4.2.

4.4.4.4 Type in the information as shown in the Report Point window (Fig 4.6).

4.4.4.5 Click OK.

4.4.4.6 Sequence Window (Fig 4.2) will be displayed.

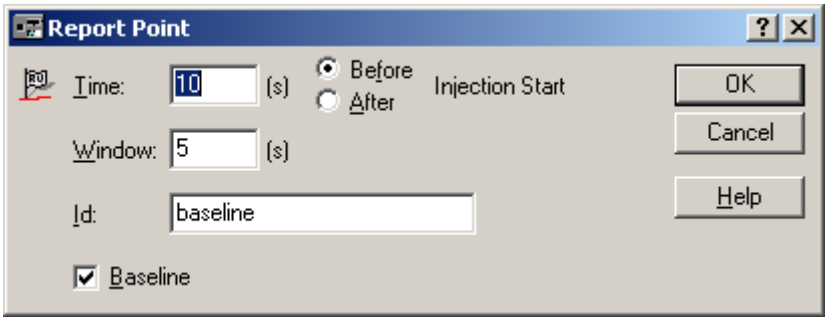


Figure 4.6

4.4.6 Step 5 – Injection of 45 □L of 1 M ethanolamine

4.4.5.1 Double click on Inject 'Ethanolamine 45'

4.4.5.2 Inject window (Fig. 4.7) will open.

4.4.5.3 Type in the information as shown in the Inject window (Fig 4.7).

ATTACHMENT 4 (Continued)

Surface Deactivation

4.4.7 Click 'OK'

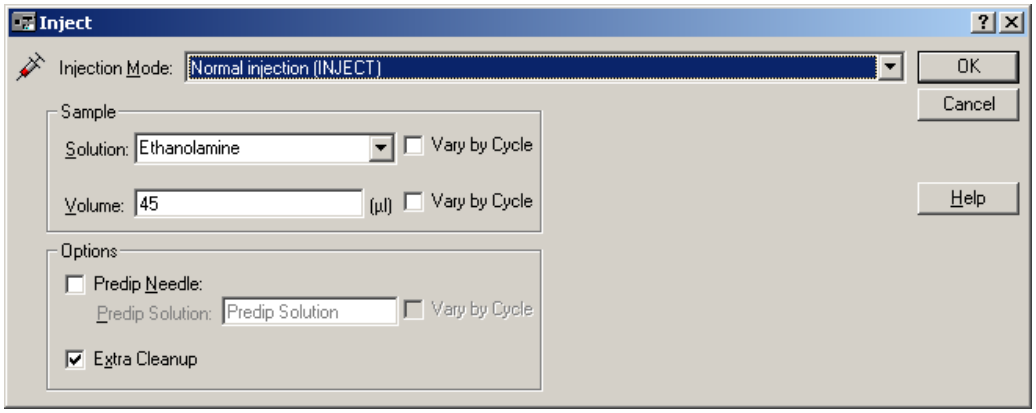


Figure 4.7

4.4.8 Sequence Window (Fig 4.2) will be displayed

4.4.9 Step 6 - Report Point on the Baseline after injection

4.4.9.1 Double click on -20 BASELINE 'Baseline_after 5'

4.4.9.2 Type in information as shown in the Report Point window (Fig. 4.8)

4.4.9.3 Click OK. Sequence window (Fig. 4.2) will be displayed.

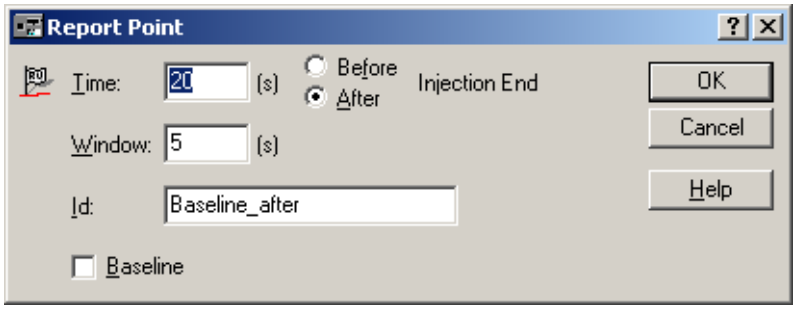


Figure 4.8

4.4.10 Click on 'Next'.

ATTACHMENT 4 (Continued)

4.4.11 Number of cycles set up

Number of cycles to be run should be set as 1 (Fig 4.9)

Click on 'Next'

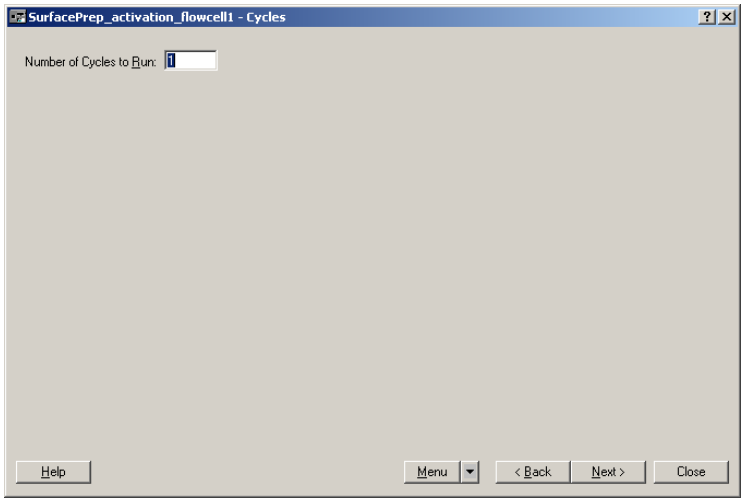


Figure 4.9

4.5 Rack Positions

4.5.1 Place 1M ethanolamine in the rack position, A1, displayed in Fig 4.10

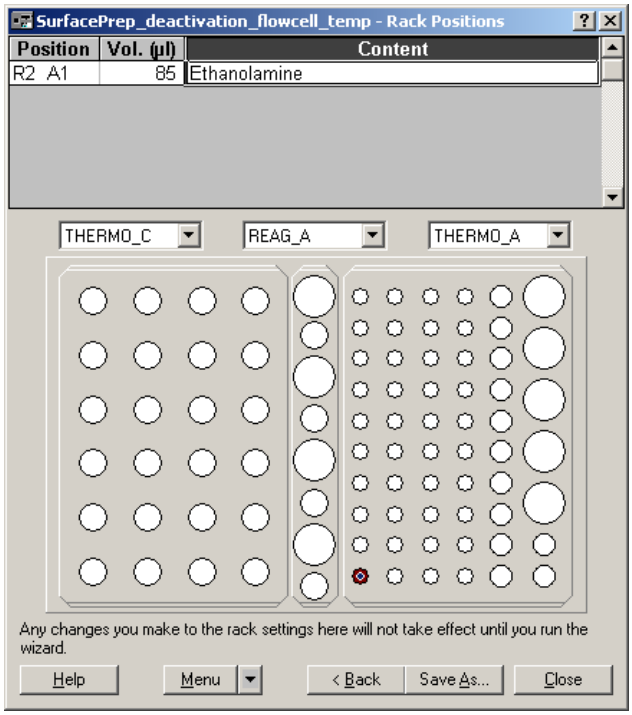


Figure 4.10

ATTACHMENT 4 (Continued)

4.5.2 Save the method file as ‘SurfacePrep_Deactivation_Flowcell1’ in the appropriate folder. (If other flow cells are being used, name the file with the corresponding flow cell number).

4.5.3 Run this method file for the activation of flow cell1 using the ‘Prepare Run’ window that will open after the step shown above. Click on ‘Start’ for initiating the procedure after selecting ‘Prime Before Run’ and ‘Standby Flow After the Run’.

ATTACHMENT 5

Kinetics

NOTE: The sensor chip CM5 has 4 flow cells that can be utilized for kinetic runs. Following configuration of flow cells can be used for kinetic runs –

In the first scenario all the 4 flow cells are used in the kinetic run. Flow cell 1 serves as the negative control and flow cells 2, 3, and 4 contains immobilized ligand. The program can be set to give sensorgrams that are subtracted from the control sensorgram.

In the second scenario flow cells 1 and 2 or 3 and 4 are utilized. In this case flow cell 1 acts as the control for flow cell 2 while flow cell 3 acts as that for flow cell 4. Each pair can be run individually or together.

The protocol given below is for the second scenario in which flow cell 1 and 2 are used. In this case flow cell 1 is the negative control surface while flow cell 2 has immobilized ligand.

For Kinetic runs, project specific templates needs to be generated and used with the project specific SOP.

Following is only an example of a kinetic run.

Start BIAcore control software

5.1 From File → Open the template file H:\5PA\PAOnly\BioAnPublic\Biacore \Templates\ Kinetics_Date_Lot# (Wizard Template window will open)

5.2 Write a brief note in the Notebook part. An example is shown in Figure 5.1

5.3 Click on Edit

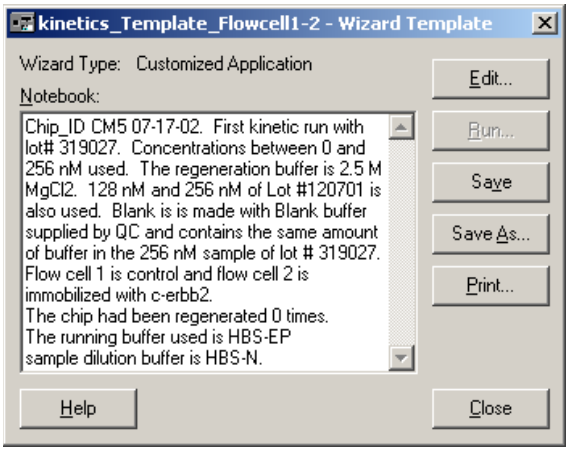


Figure 5.1 (This is only an example of a note; the analyst or operator will enter a specific note consistent with the experiment).

ATTACHMENT 5 (Continued)

Kinetics

5.4 Sequence window shown figure 5.2 will open.

The parameters that need to be changed are 'DETECTION' (Step 1) and FLOWPATH (Step 4). In the example shown in fig 5.2, 'DETECTION' is selected as 2-1 and 'FLOWPATH' as1, 2. (Flow cells 1 and 2 in which 1 is serving as the control).

5.5 Click on 'Next'

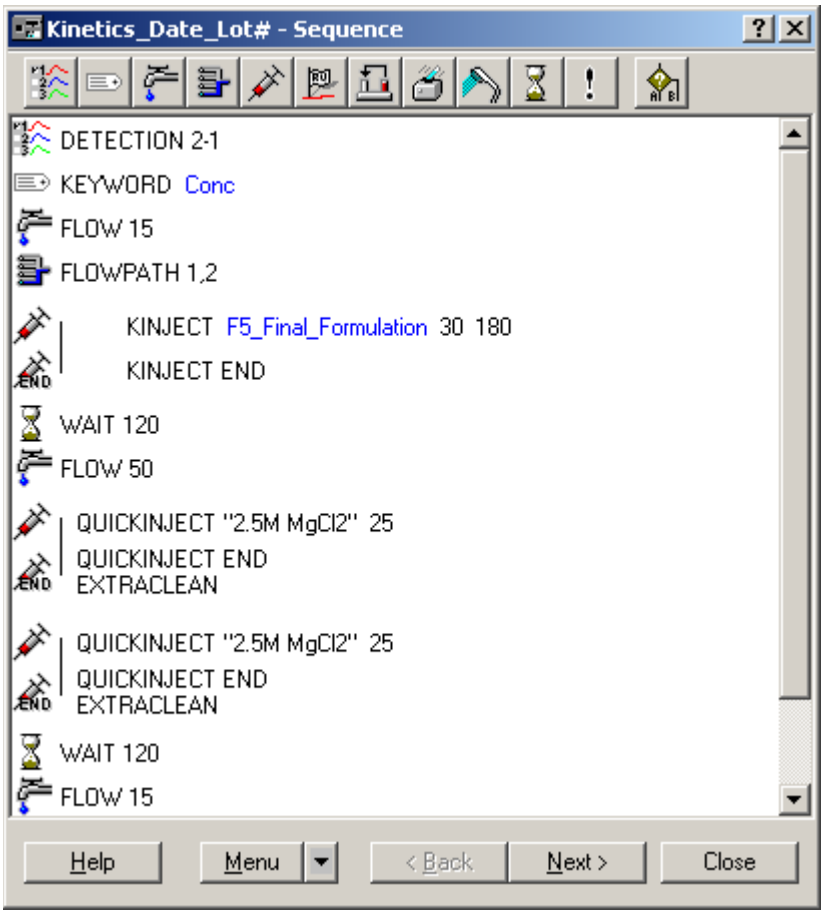


Figure 5.2

5.6 The Cycles window (figure 5.3) will open.

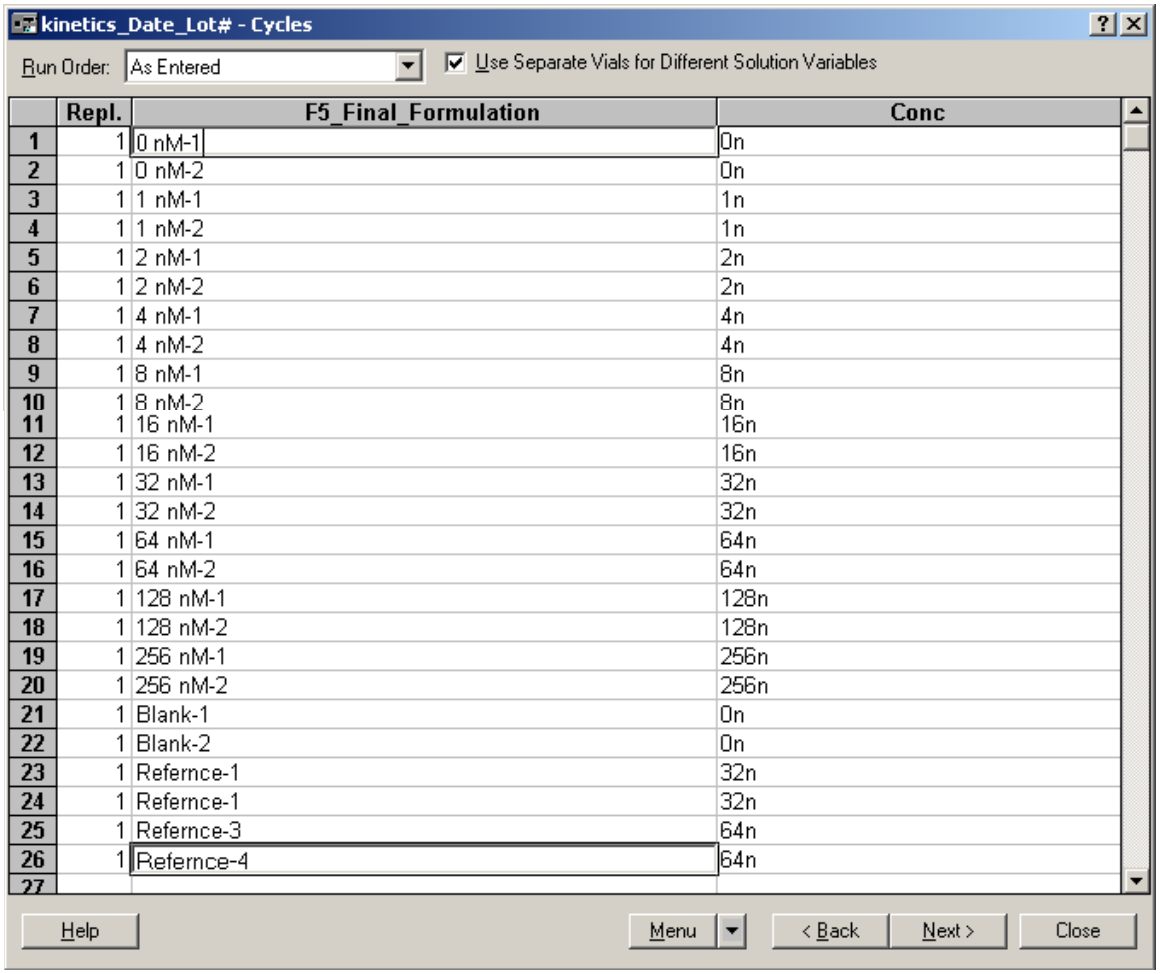
This window contains the information of the various concentrations of sample in duplicate and two concentrations of the reference lot (32 nM and 64 nM), also in duplicate. 'n' in the Conc column denotes nano molar.

5.7 Click on 'Next'.

ATTACHMENT 5 (Continued)

Kinetics

5.8 Rack positions window (Figure 5.4) will open.



	Repl.	F5_Final_Formulation	Conc
1	1	0 nM-1	0n
2	1	0 nM-2	0n
3	1	1 nM-1	1n
4	1	1 nM-2	1n
5	1	2 nM-1	2n
6	1	2 nM-2	2n
7	1	4 nM-1	4n
8	1	4 nM-2	4n
9	1	8 nM-1	8n
10	1	8 nM-2	8n
11	1	16 nM-1	16n
12	1	16 nM-2	16n
13	1	32 nM-1	32n
14	1	32 nM-2	32n
15	1	64 nM-1	64n
16	1	64 nM-2	64n
17	1	128 nM-1	128n
18	1	128 nM-2	128n
19	1	256 nM-1	256n
20	1	256 nM-2	256n
21	1	Blank-1	0n
22	1	Blank-2	0n
23	1	Refernce-1	32n
24	1	Refernce-1	32n
25	1	Refernce-3	64n
26	1	Refernce-4	64n
27			

Figure 5.3

NOTE: The concentration, sample details, and name will need to be edited to be consistent with the experiment that will be run.

5.9 Prepare appropriate concentrations of the product in 1.5 mL eppendorf tube. (Details of sample preparation will be captured in the project specific form (e.g., 16139-01)

5.10 Place 80 µL of each sample (duplicates of each concentration is kept in separate vials) in 7 mm polypropylene vials displayed in figure 5.4.

ATTACHMENT 5 (Continued)

Kinetics

5.11 Also place appropriate amount of regeneration buffer (e.g., 2M MgCl2) in a 16 mm glass vial in the rack position displayed in fig. 5.4.

5.12 Then save the method file in the working directory as 'Kinetics_Date_Lot#'.

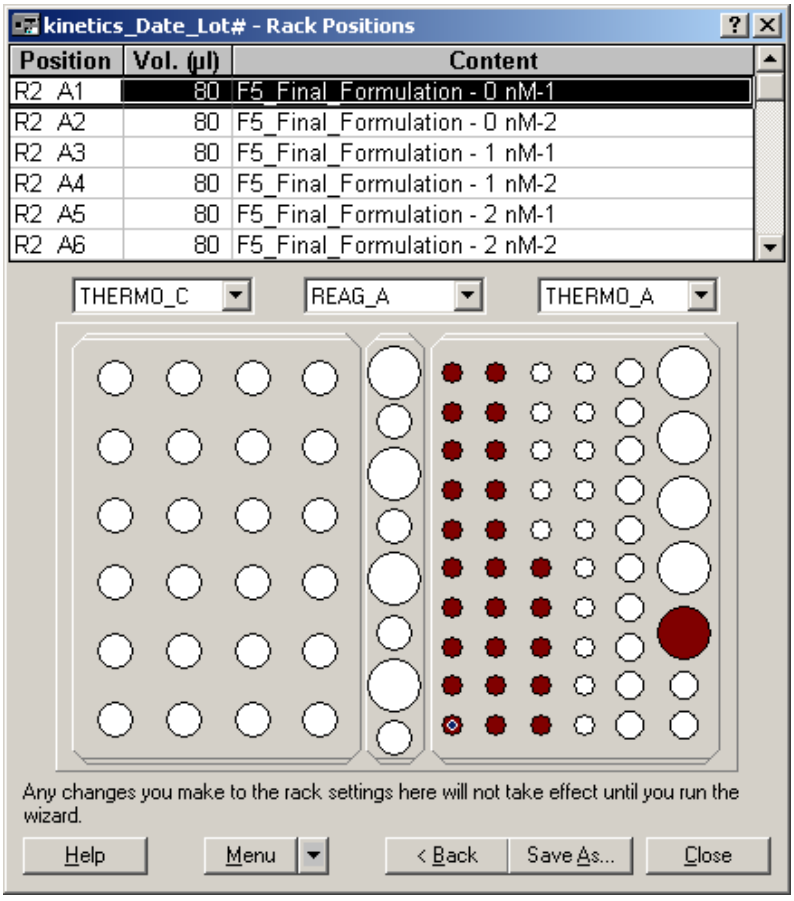


Figure 5.4 (Note: The sample name and concentration should be consistent with the experiment that will be performed)

5.13 Open this method file for starting the kinetic run using the 'Prepare Run' window that will open after the step shown above. Click on 'Start' for initiating the procedure after selecting 'Prime Before Run' and 'Standby Flow After the Run'.

5.14 The program will prompt for a file name to save the results of the kinetic run and general format for the results file name 'Results_Kinetics_Date_Lot#'. Save this file in the working directory.

ATTACHMENT 6

Data Analysis

6.1 Open the program BIAevaluation 3.1.

6.2 Open the result file named 'Results_Kinetics_Date_Lot#' from the working directory.

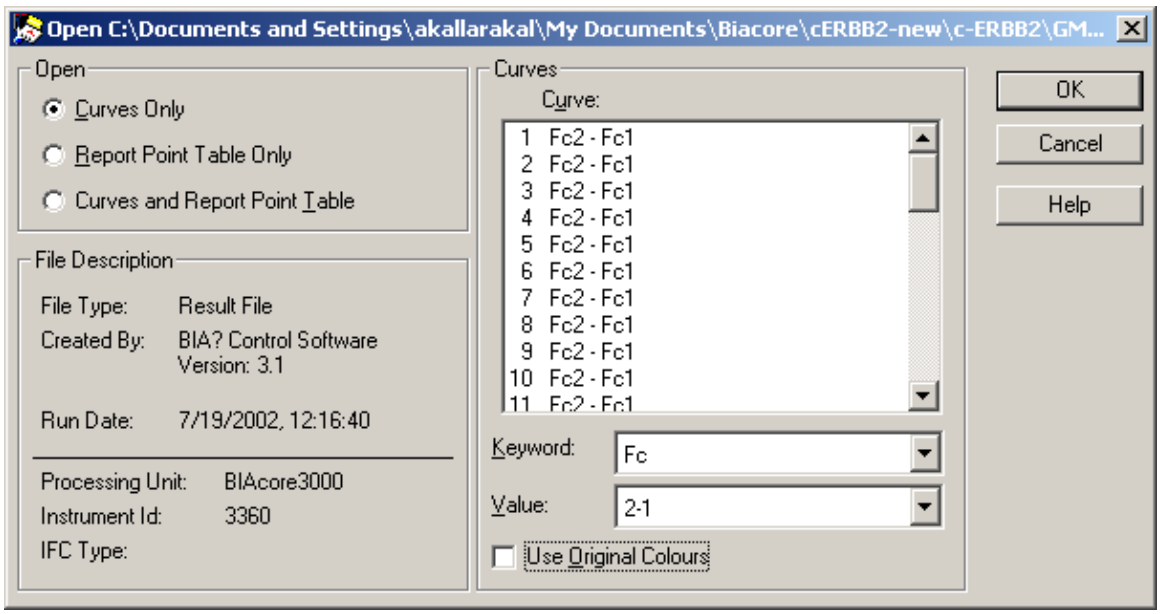


Figure 6.1 (Note: check that the sample name and details reflect the experiment performed)

6.3 Window shown in Fig 6.1 will open.

6.4 Select 'Curves Only', Keyword 'Fc', and Value '2-1' (selects data from flow cell 2. If data from flow cells 3 or 4 needed, select 3-1 or 4-1). Click OK

6.5 Window shown in Fig 6.2 will open with the list of sensorgrams.

6.6 Click 'OK'.

6.7 Window shown in Fig 6.2 will open with the list of sensorgrams.

6.8 Select the required curves by holding down the left mouse key. (Holding down the Ctrl key and then "Clicking" with the mouse can be used to select curves that are not in order as listed on the screen). Initially select the first 22 curves that represent the sensorgrams for the various concentrations (0 – 256 nM) of the sample.

6.9 Click on 'Overlay Plots' button (Fig 6.2) and window Fig. 6.3 opens.

ATTACHMENT 6 (Continued)

Data Analysis

6.10 Window shown in Fig 6.3 will open displaying the overlaid sensorgrams.

'**Overlay Plots**' Button

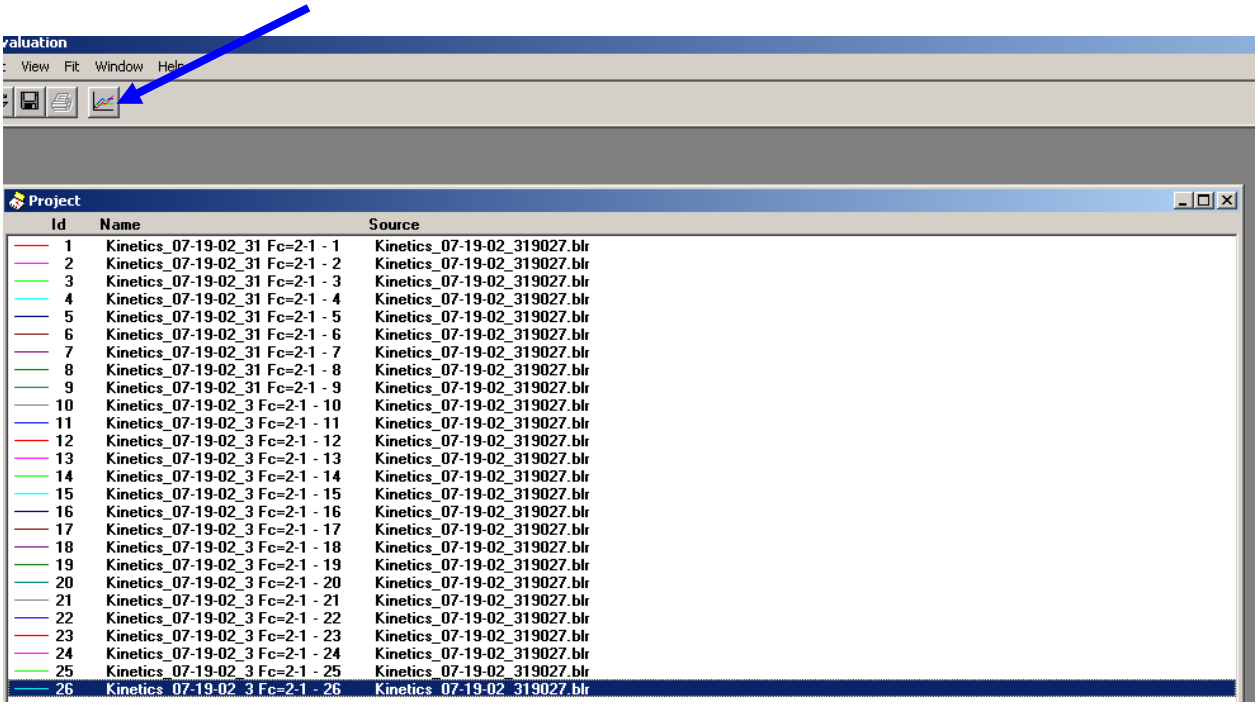


Figure 6.2 (Analyst should verify the entry)

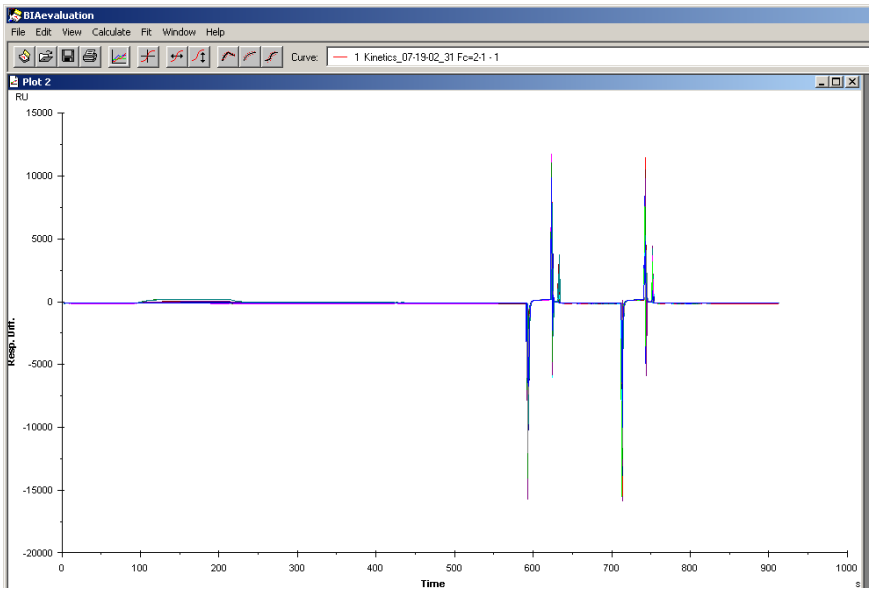


Figure 6.3

ATTACHMENT 6 (Continued)

Data Analysis

6.11 Holding down the right mouse button select the region (regeneration) shown in Fig 6.4

6.12 Remove this region of the sensorgrams using the command ‘Cut’ under ‘Edit’.

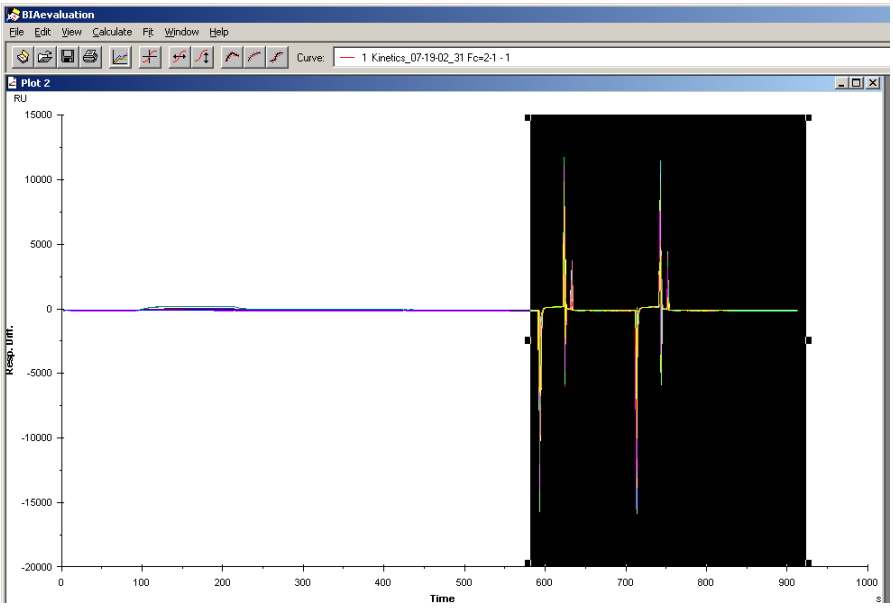


Figure 6.4

6.13 The resulting sensorgrams are shown in Fig 6.5

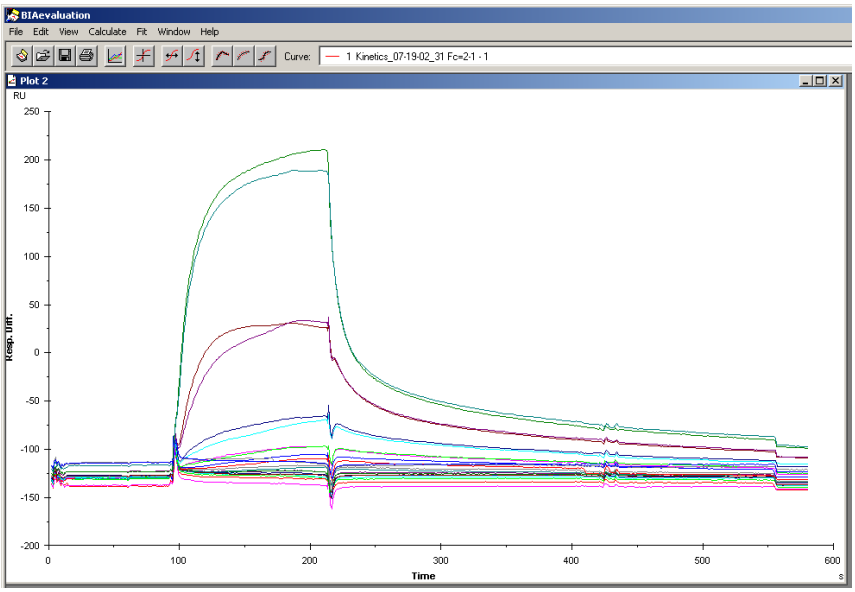


Figure 6.5

ATTACHMENT 6(Continued)

Data Analysis

6.14 Hold down the right mouse key and draw a bar on the region of the sensorgrams as shown in Fig. 6.6 (The region that is selected is that just before the injection).

6.15 Click on Y-transform

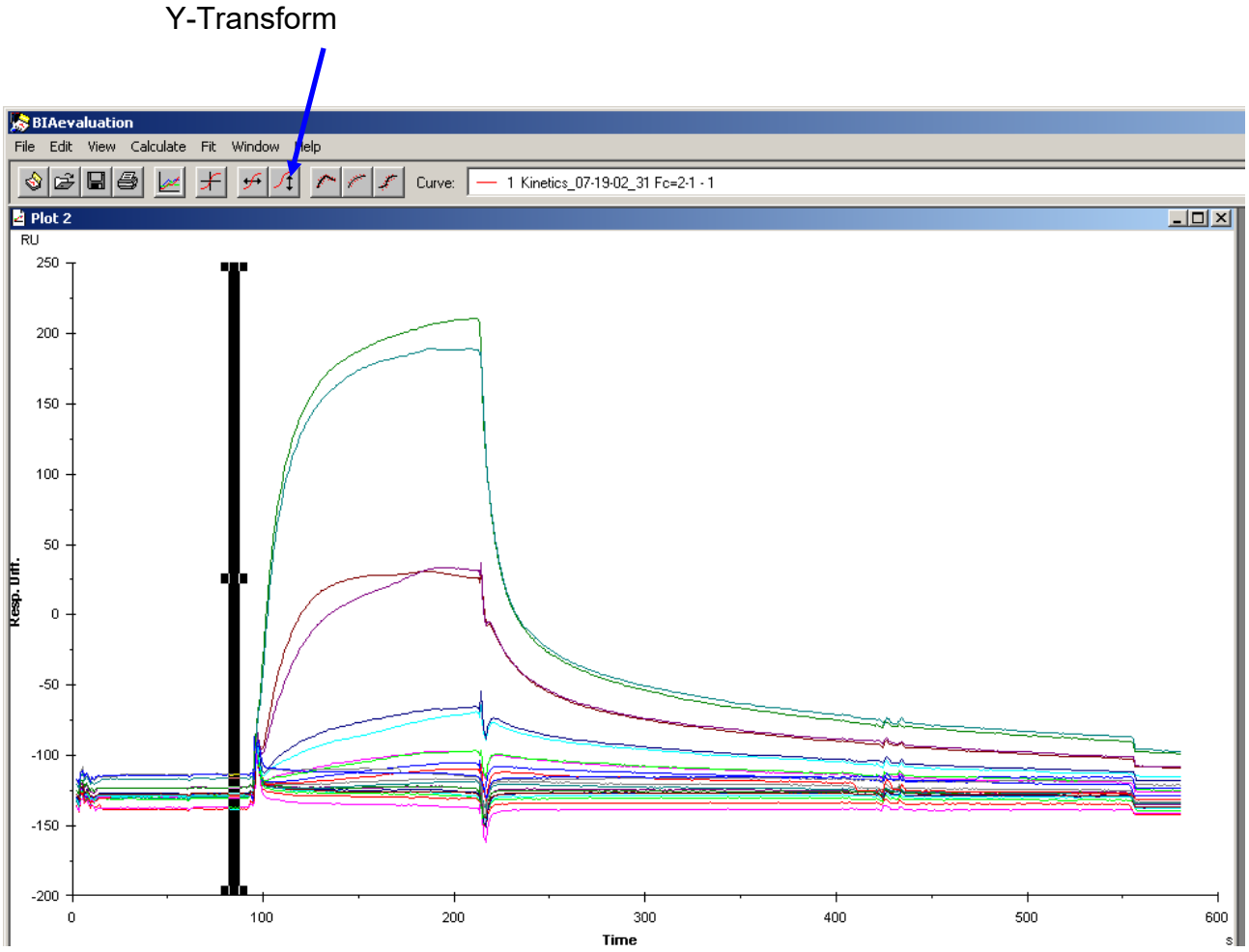


Figure 6.6

ATTACHMENT 6 (Continued)

Data Analysis

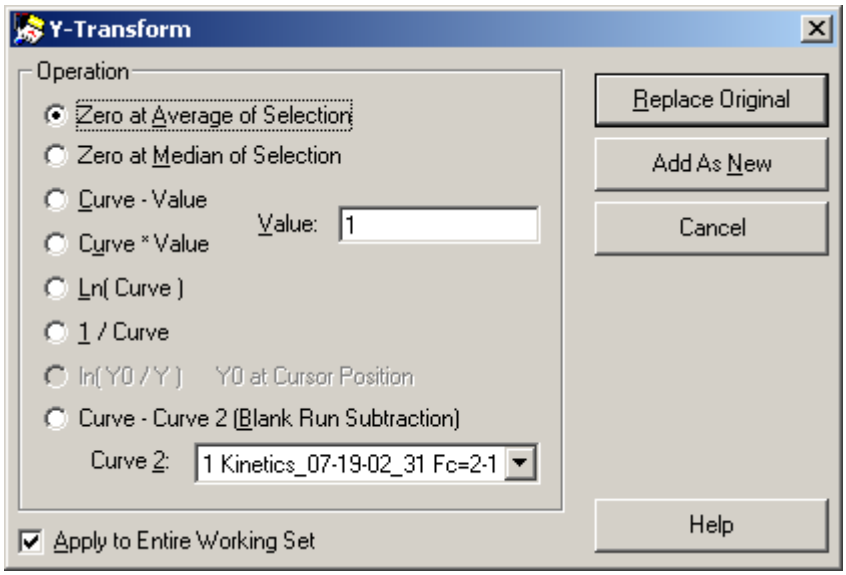


Figure 6.7

6.16 Y-transform window will open (Fig. 6.7).

6.17 Select ‘Zero at Average of Selection’ (Fig. 6.7).

6.18 Click on ‘Replace Original’.

6.19 Click on Y-transform button again.

6.20 Select Curve – Curve 2 (Blank Run Subtraction).

6.21 Select Sensorgram for the Blank run (Curve 2 in Fig 6.7).

6.22 Click on ‘Replace Original’.

ATTACHMENT 6 (Continued)

Data Analysis

'Fit Kinetics, simultaneous ka/kd' Button

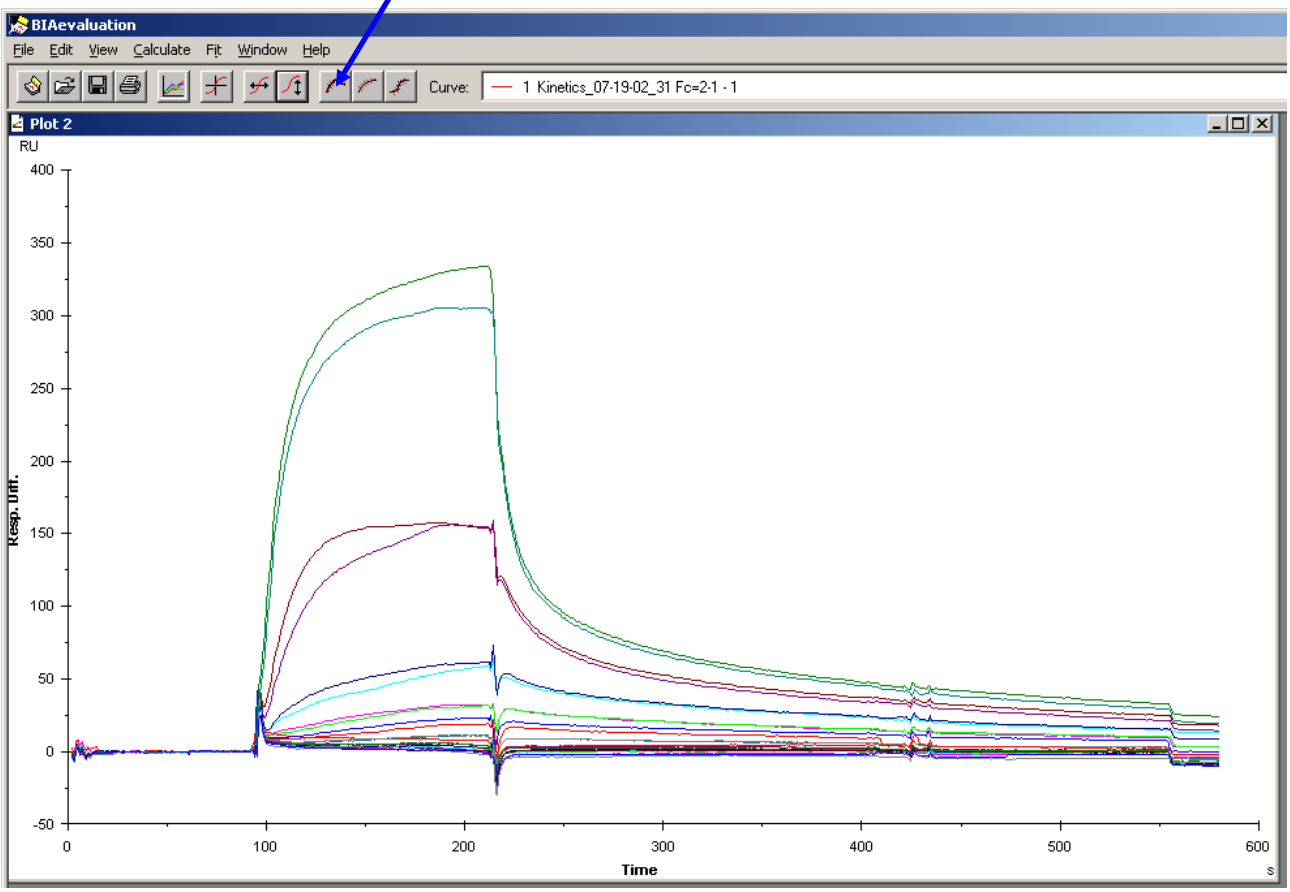


Figure 6.8

6.23 Window shown in fig 6.8 with sensorgrams normalized on the Y- axis will open.

6.24 Click on 'Fit Kinetics, simultaneous ka/kd' button (Fig 6.8).

6.25 'Fit Kinetics, simultaneous ka/kd' window (Fig 6.9) will open.

6.26 Click on 'Next' (Fig 6.9).

6.27 Data selection window will open (Fig 6.10).

6.28 Select the data using the markers (Injection start, Association, Injection stop and Dissociation) on selection bar (Fig 6.10).

ATTACHMENT 6 (Continued)

Data Analysis

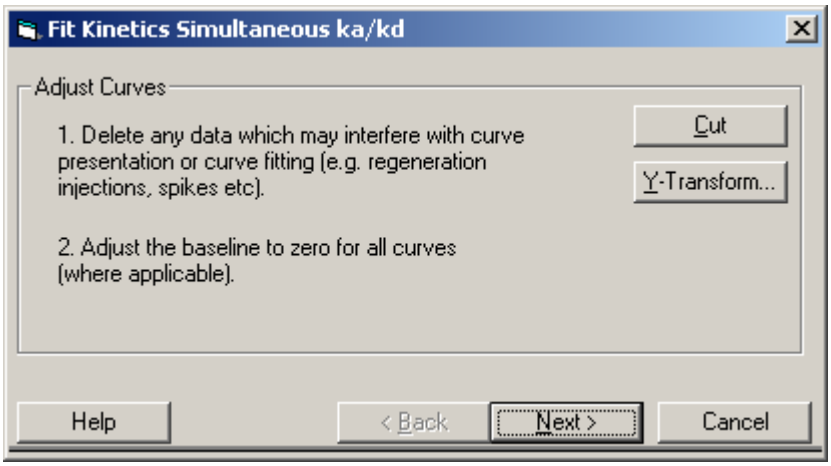


Figure 6.9

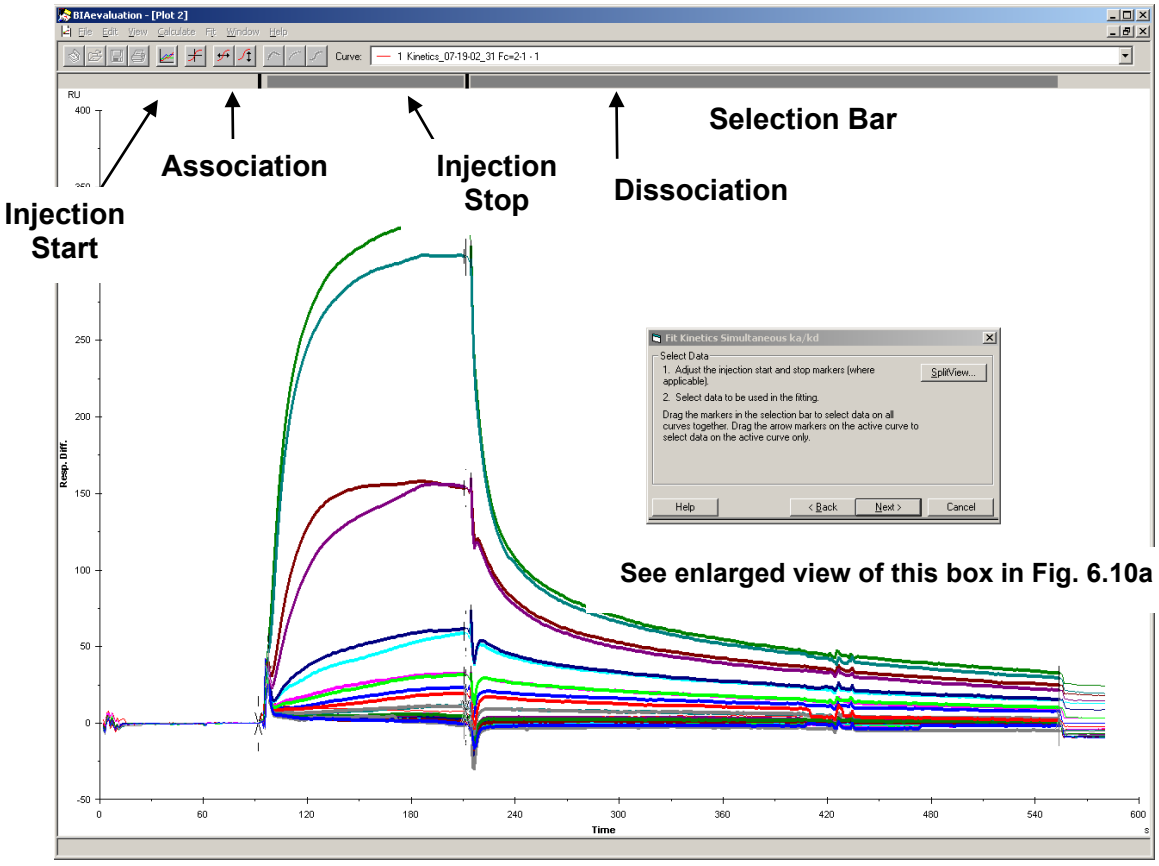


Figure 6.10

Inset

ATTACHMENT 6 (Continued)

Data Analysis

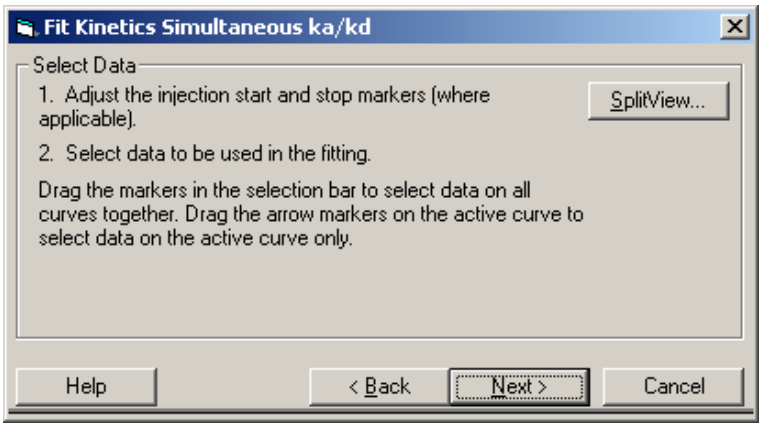


Figure 6.10a (Fig 6.10 inset)

6.29 Click 'Next' (Fig 6.10a)

6.30 Data fit window (Fig 6.11) will open

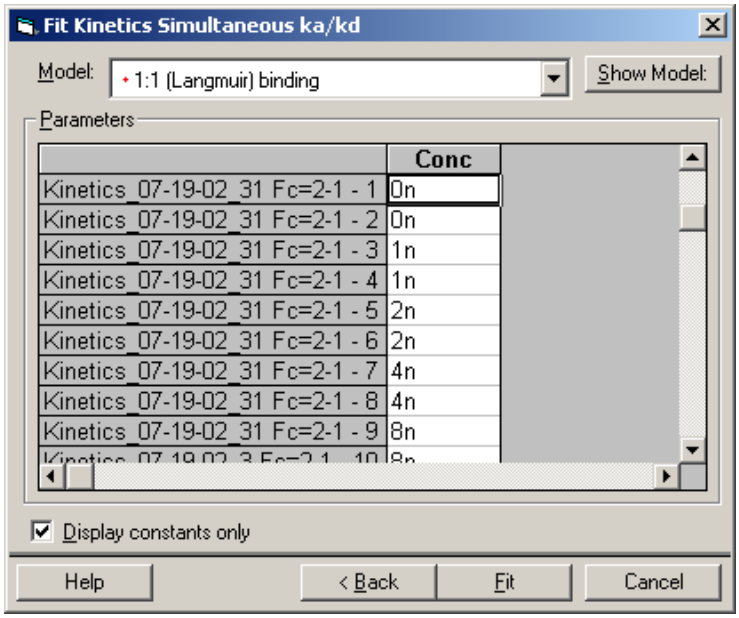


Figure 6.11

6.31 Select '1:1 [Langmuir] binding' as the Model for fitting the data

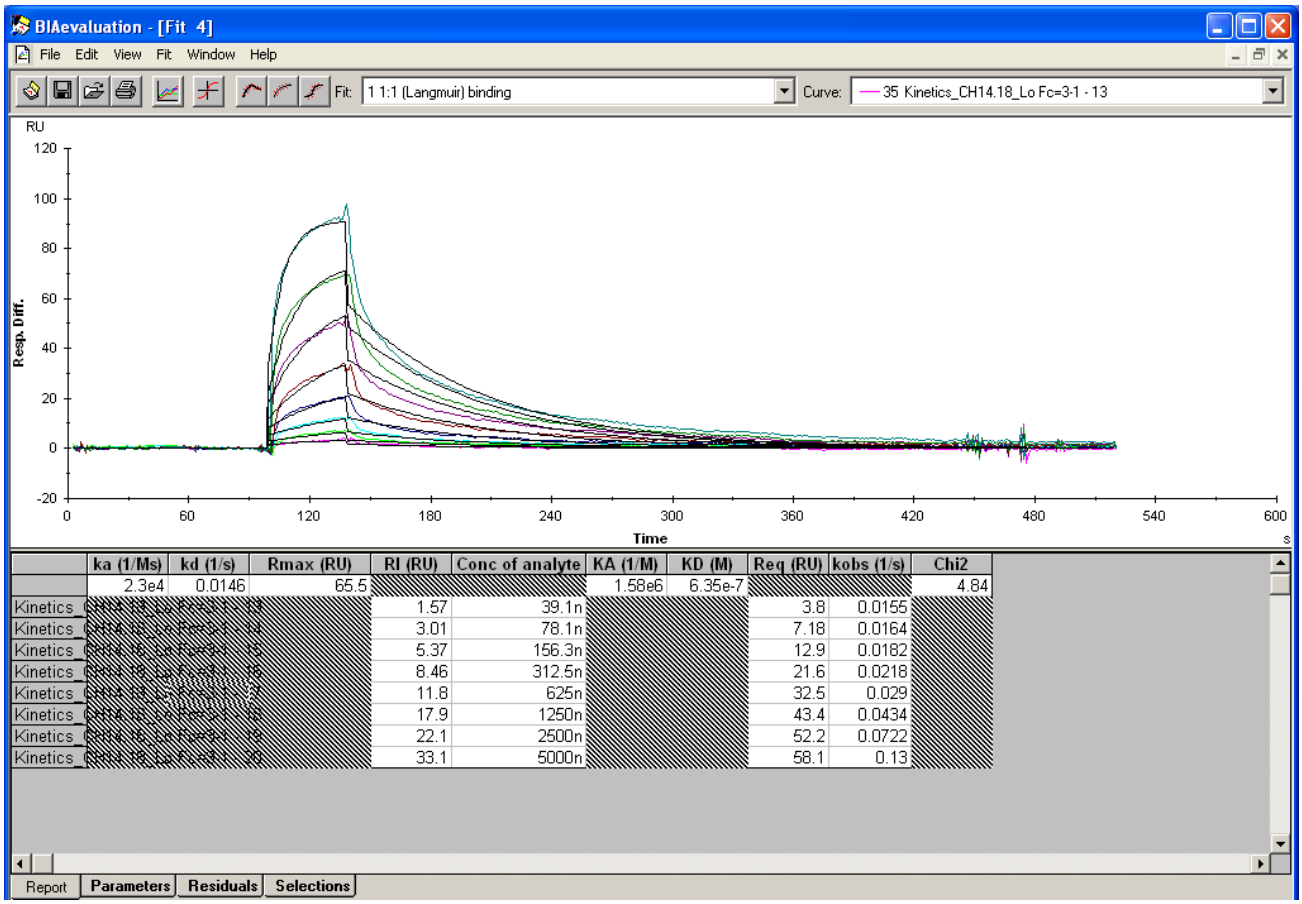
6.32 Click on 'Fit'.

ATTACHMENT 6 (Continued)

Data Analysis

6.33 Report the kinetic parameters ka, kd, KA, and KD obtained by the global fitting of the data using 1:1 [Langmuir] binding as the model.

NOTE: Fig 6.12 shows an example of the results page after analysis of the kinetic data

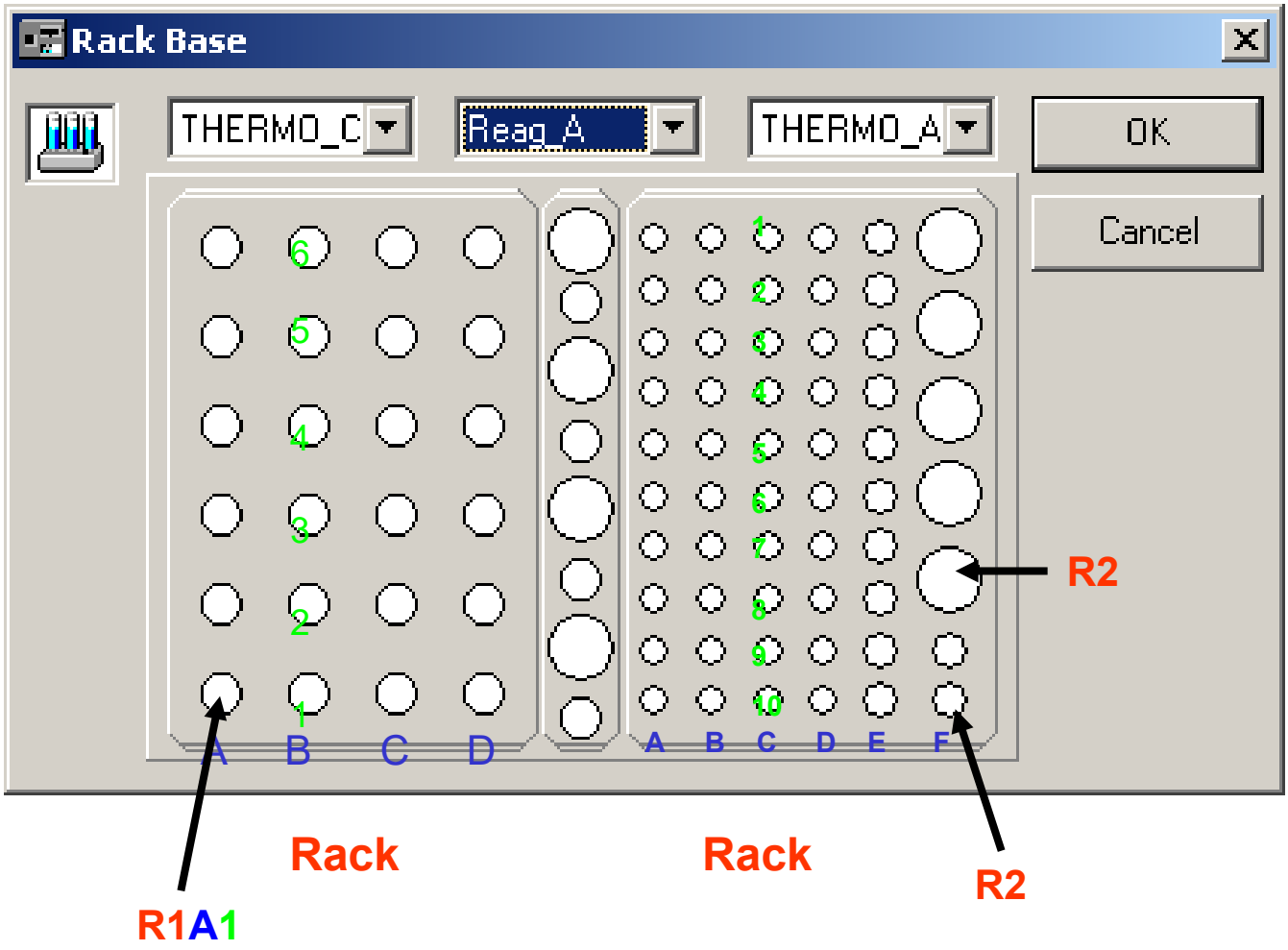


	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Conc of analyte	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	2.3e4	0.0146	65.5			1.58e6	6.35e-7			4.84
Kinetics_CH14.18_Lo Fc=3-1 - 13				1.57	39.1n			3.8	0.0155	
Kinetics_CH14.18_Lo Fc=3-1 - 14				3.01	78.1n			7.18	0.0164	
Kinetics_CH14.18_Lo Fc=3-1 - 15				5.37	156.3n			12.9	0.0182	
Kinetics_CH14.18_Lo Fc=3-1 - 16				8.46	312.5n			21.6	0.0218	
Kinetics_CH14.18_Lo Fc=3-1 - 17				11.8	625n			32.5	0.029	
Kinetics_CH14.18_Lo Fc=3-1 - 18				17.9	1250n			43.4	0.0434	
Kinetics_CH14.18_Lo Fc=3-1 - 19				22.1	2500n			52.2	0.0722	
Kinetics_CH14.18_Lo Fc=3-1 - 20				33.1	5000n			58.1	0.13	

Fig 6.12 Results of a kinetic analysis showing association constant ka, dissociation constant kd, and equilibrium dissociation constant K_D along with other parameters.

ATTACHMENT 7

Rack Positions



Rack Bases and Positions

R1 rack takes 9 mm glass vials (BDP PN 22076)
R2 rack positions R2 A1 – R2 F2 take 7 mm plastic vials (BDP PN 22064) and positions R2 F3 – R2 F7 takes 16 mm glass vials (BDP PN 22066)