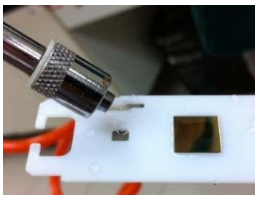
Biacore T200 Start up/Shut down Protocol Simplified

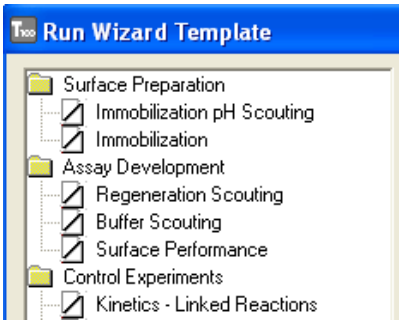
STOP This protocol is for qualified users operating Biacore T200 in IBC 402 only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's handbooks is highly recommended.

Start up procedure:



1. All buffers and solutions for use in the system should be filtered through a 0.22 μm filter.
2. Place your buffer at left buffer tray (tube A) and ddH_2O at right waste and water tray.
3. Fasten the clamp of the peristaltic pump in the right pump compartment.
4. Turn on the instrument and control PC.
5. Start Biacore T200 control software.
6. When the chip port cover opens automatically, put a ***maintenance chip***, close the port cover and **Dock** the chip.
7. Run **Tools: Prime** to fill the flow system with your buffer. When done, choose **Tools: Eject Chip**, replace the maintenance chip with your chip***** and click **Dock Chip** to dock the chip on to IFC.

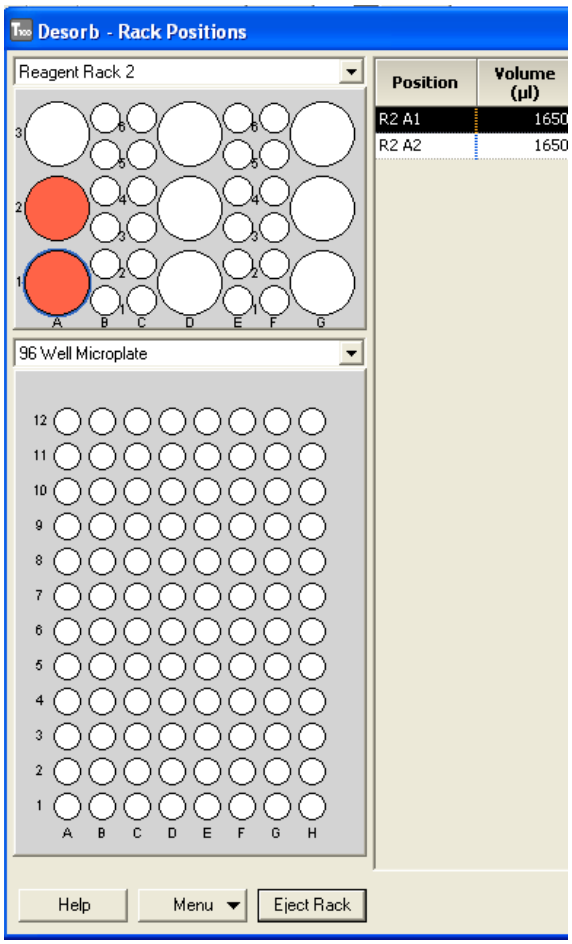
   ***** Please note that wet chips will contaminate the optical window and damage the insert band. If your chip has been stored wet in buffers, please rinse only the glass side of the sensor with ddH2O and blow dry with air. Wipe the plastic sensor holder dry with Kimwipes before inserting the sensor to the chip cassette.
8. Choose **Tools: Eject Rack** to load samples to the rack tray**.** Please note that the rack tray automatically moves into the sample compartment 60 seconds after it has been eject.
9. There are 3 modes to set up your experiments, **Manual run**, **Wizard** and **Method**. To start a wizard run, choose **Run: Wizard**. You may edit an old one or make a new template from four different categories of default templates. For more options, such as multi detection mode or single cycle kinetics, choose **Run: Method** to edit and run an experiment.

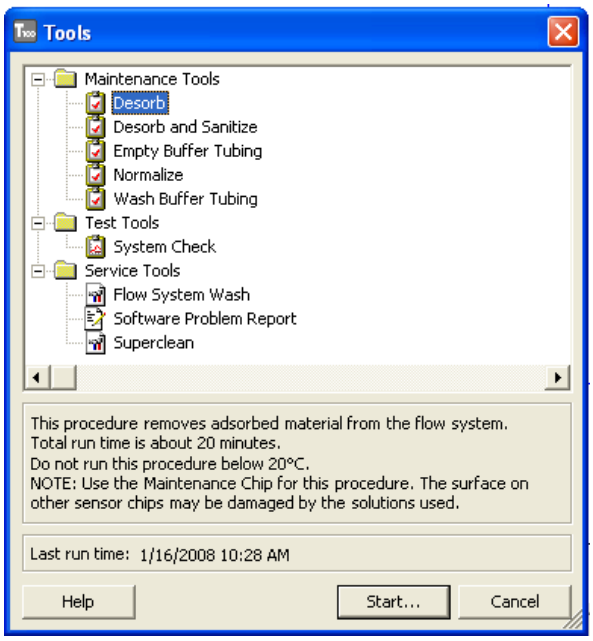


10. When a run is complete, the instrument is automatically switched to standby mode. It uses about 65 mL/day of buffer A. If you were to leave your sample chip overnight, you may just left the instrument at standby mode.

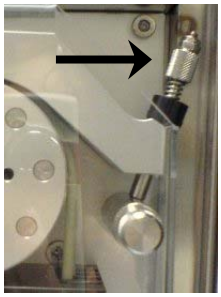
Shut down procedure:

11. Choose **Tools: Eject Chip** (or **Tools: Insert Chip**) to open the sensor chip port cover. Take out your chip and place ***maintenance chip*** to the chip port. Close the port cover by pressing the cover. Rinse tubings with ddH2O and replace buffer A with ddH_2O. **Select "⊙new chip" maintenance chip** and Click **Dock Chip** to dock the chip on to IFC.
12. Run **Tools: Prime** to fill the system with water.
13. Under **Tools: More Tools,** choose **Maintenance Tools: Desorb** and load the sample to rack tray according to the pop up dialog box. It takes 1650 μL for both BIAdesorb solution 1 (0.5% SDS) and solution 2 (50mM, pH 9.5 glycine). The process takes about 20 minutes.
14. **Eject rack tray** when done.
15. Choose **Tools: Shutdown** to flush the flow system and empty the IFC and tubes. It takes about 20 minutes and required water and 45 mL of 70% ethanol. Please follow the pop up instructions step by step.





16. Switch off the instrument. Open the right pump compartment and flip the tube clamp to the right.



17. Empty water bottles and waste bottle. Insert solvent lines to empty bottles.
18. Turn off control PC and monitor.

Experiment optimization procedures for chip CM5:

-> Nonspecific binding test -> pH Scouting -> Immobilization ->
Single Cycle Kinetics -> Optimize assay condition ->
Find Regeneration Buffer ->Multiple Cycle Kinetics

a. Start a manual run to test for possible nonspecific binding of analyte to the CM5 sensor surface. Use running buffer to dilute your analyte and inject through the second channel. Check if there is any time dependent response or slow-off kinetics. After injection, wash and regenerate sensor surface with 50mM NaOH. Optimize buffer compositions if nonspecific binding occurs.
b. Start a manual run or a wizard of pH scouting to find optimal pH for immobilization. Dilute your ligand to a final concentration of 10ug/mL in pH 4.5, 5.0, 5.5 or 6.0 10 mM sodium acetate buffers. Inject each solution sequentially through the second channel and look for suitable binding slope of your ligand to the sensor surface. Usually the pH is within the range of pH3.5 to the pI of your ligand minus 1. The immobilization level is determined so that the Rmax is less than 250 RU for kinetics study. Solution for immobilization should not contain primary amines (for examples, Tris buffer or gentamicin) or high concentrations of salt. After injection, wash and regenerate surface with 50mM NaOH.
c. Start an immobilization wizard to fix your ligand to the second (or fourth) channel using the acidic buffer found in the previous step as the immobilization buffer. The first (or third) channel may be treated by activating blank immobilization option in the same wizard.
d. Start single cycle kinetics (SCK) method using running buffer as the regeneration buffer and run 4 sample cycles (+ 3 start-up cycles) with high and low concentration series, such as follows:

	Sample solution	Conc(1)	Conc(2)	Conc(3)	Conc(4)	Conc(5)
1	Analyte	0	0	0	0	0
2	Analyte	0	0	0	0	0
3	Analyte	0.23	0.69	2	6.17	18.5
4	Analyte	18.5	55.5	167	500	1500

e. Analyze SCK results and optimize concentration ranges for next kinetics experiment after regeneration buffer is known.
f. Start a manual run to look for regeneration buffers.
g. Set up a multiple cycle kinetics using Wizard if the surface can be regenerated.