Biacore T200 Single-Cycle Kinetics Protocol

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.

1. In this protocol, we will use the Biacore single-cycle kinetics (SCK) method to obtain kinetics data, using samples from Biacore T200 Getting Started kit. Choose **Run:Method**. In the folder **Methods And Templates**, select **Biacore Methods**. Double click **Single-cycle kinetics** to open the default setting for SCK.
2. Check the **Overview**. The overview shows a summary of the method.
3. Click the **General Setting** and set the parameters as followed:

   Data collection rate: 10Hz,

   Detection mode: Dual (choose “Multiple” if all 4 channels are needed),

   Temperature: 25,

   Concentration unit: nM

   Buffer A: HBS-EP+
4. Click the **Assay Steps** and select the steps to alter the setting.

   Select the **Startup** and change the **Number of replicates** to 3. No changes for the **Sample** step.
5. Click the **Cycle Types** and select the **Sample** cycle. The cycle is executed sequentially according to the commands. Under **Commands** panel, select **Sample 1** and set the parameters as followed:

   Type: Single cycle kinetics

   Concentration per cycle: 5

   Contact time: 120 s

   Dissociation time: 600 s

   Flow rate: 30 μL /min

   Flow path: Both

   Under **Commands** panel, select the **Regeneration 1** and set the parameters as followed:

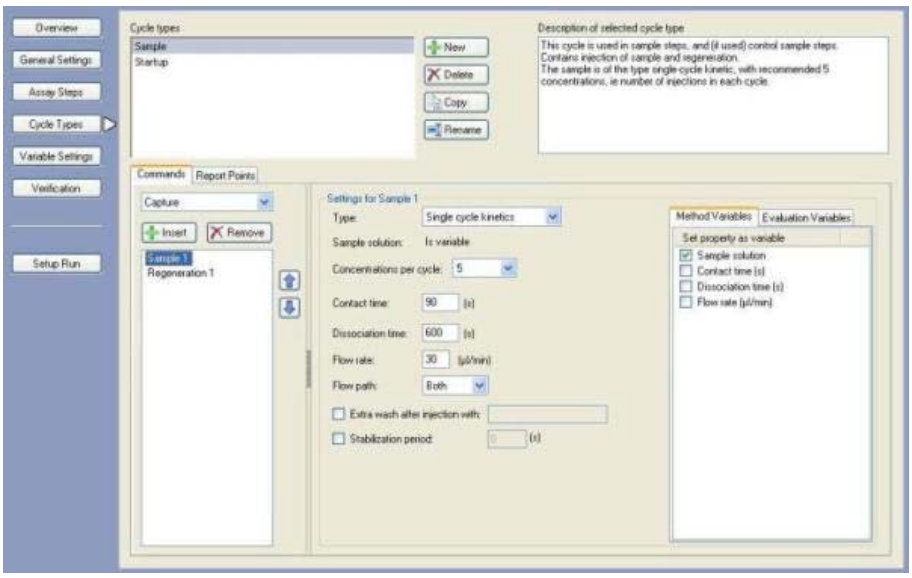
   Regeneration solution: 10mM Glycine pH2.5

   Contact time: 30s

   Flow Rate: 30 μL /min

   Flow path: Both

   Stabilization period: 30s



6. Still under the **Cycle Types**, select the **Startup** cycle. Under **Commands** panel, select **Sample 1** and set the parameters as followed:

   Type: Low sample consumption

   Contact time: 120 s

   Dissociation time: 120 s

   Flow rate: 30 µL /min

   Flow path: Both

   Under **Commands** panel, select the **Regeneration 1** and set the parameters as followed:

   Regeneration solution: 10mM Glycine pH2.5

   Contact time: 30s

   Flow Rate: 30 µL/min

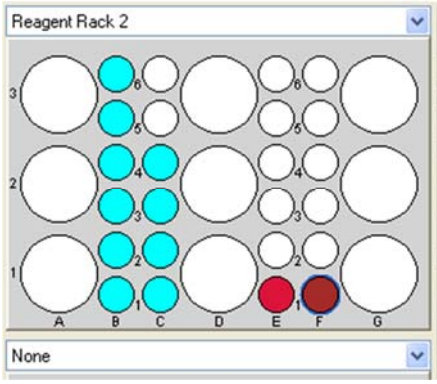
   Flow path: Both

   Stabilization period: 30s

7. Click on **Variable Settings**. Check ⊙ Define all values at run time for both Startup and Sample steps.
8. Click on **Verifications** to verify the method.
9. Click on **Setup Run**. Choose **Flow path**. Click on **Next**. Use HBS-EP+ as **Startup** solution. Select **Sample** and type in *beta2-microglobulin* as the sample solution and the concentrations are *0* nM, *0* nM, *0* nM, *0* nM and *0* nM in the first and second cycles and *2* nM, *4* nM, *8* nM, *16* nM and *32* nM in the third. The **MW** of beta2-microglobulin is *11800* Da. Click on **Next** when done.
10. The **Cycle run list** shows a summary of the experiment cycles. Click on **Next**.

11. In **System Preparations**, uncheck **Prime before run** as well as **Normalize detector**. Click on **Next**.

12. Prepare your samples: Dilute stock beta2-microglobulin in running buffer to 32 nM (2.26 µL beta2-microglobulin + 598 µL HBS-EP). Prepare the concentration series from the 32 nM sample: mix 300 µL of the 32 nM solution with 300 µL running buffer to get the 16 nM solution. Continue the dilution series to obtain the following: 32, 16, 8, 4 and 2 nM.



Position	Volume (µl)	Content	Type	Sample 1 Conc (nM)	Sample 1 MW (Da)
R2 F1	320	Glycine pH2.5	Regeneration		
R2 E1	154	HBSEP+	Startup		
R2 C4	118	beta2-microglobulin	Sample	32	11800
R2 C3	118	beta2-microglobulin	Sample	16	11800
R2 C2	118	beta2-microglobulin	Sample	8	11800
R2 C1	118	beta2-microglobulin	Sample	4	11800
R2 B6	118	beta2-microglobulin	Sample	2	11800
R2 B5	118	beta2-microglobulin	Sample	0	11800
R2 B4	118	beta2-microglobulin	Sample	0	11800
R2 B3	118	beta2-microglobulin	Sample	0	11800
R2 B2	118	beta2-microglobulin	Sample	0	11800
R2 B1	118	beta2-microglobulin	Sample	0	11800

13. Prepare and position samples according to **Rack Positions**. Click on **Next**.

14. Make sure everything is correct according to the **Prepare Run Protocol** and click **Start** to begin the experiment.

15. Enter a file name for the resulting sensorgram.

16. Evaluate result using Biacore T200 evaluation software.

Results of 2009/02/25:

	Multi-Cycle	Single-Cycle
k_a (1/Ms)	1.03E+6	9.10E+5
k_d (1/s)	0.0030	0.0028
K_D (1/M)	3.0E-9	3.1E-9