PRODUCT INSERT

Gator™ Flex SA Kit

Catalog No. 350001

Overview

Gator™ Flex SA Kit consists of regenerable streptavidin probes and regeneration reagents. The kit is useful for kinetic characterization of biotinylated proteins. Following immobilization of a biotinylated protein, users can quantitate k_{on}, k_{off}, and K_D of the binding interaction between the immobilized protein and its specific antibody / ligand. The proprietary surface chemistry allows the probe to be reused, thus cutting down the cost per sample by 5-10-fold depending on the number of times a probe is regenerated. If desired, different proteins can be loaded on to the probe after each regeneration, thus eliminating the need to restrict a probe to any one protein.

Materials required

Flex SA Kit	Catalog No. 350001
Max Plate	Catalog No. 130062
Black Plate	Greiner 655209
DMSO	User supplied

Storage

Store Flex SA probes (Catalog number: 160015) at room temperature in the foil pouch, ensuring zipper is fully sealed to avoid humidity/moisture contamination. In high-humidity environments, storage inside a dry cabinet is recommended. Store Flex SA Reagent Set (Catalog number: 190004) at 4°C. Reagents are stable at 4°C for 5 months.

General Applications

Kinetic studies of biotinylated proteins

General Methods

Sample Volume

Black Plate: 250 µL (200 µL minimum)
Max Plate: 300 µL

Pre-wet Conditions

260 µL Flex SA Priming Reagent in Max Plate
5 min at 1000 rpm

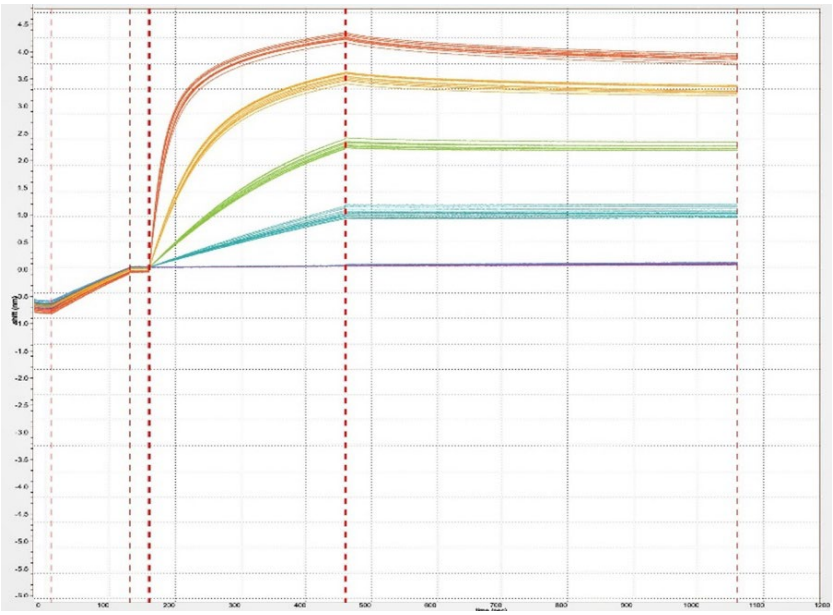

Figure 1: Following a 5 min 1000 rpm pre-wet in K buffer and capture of Flex SA Capture Reagent (SA-CR), biotinylated TNF-α was loaded onto the sensor, then exposed to association and dissociation of 10, 30, 100 and 300 nM anti TNF-α antibody. Global-fit analysis using Gator Bio™ software for the TNF-α binding interaction with anti- TNF-α resulted in K_D = 7.63E-10 M.

Notes:

1. Each probe can be reactivated up to 10 times or more. Number of reactivations are protein dependent.
2. Used probes should be stored at 4°C dipped in Priming buffer if being saved for further reactivations.
3. Reactivation plates (Max plates) may be used for a maximum of 20 dips and can be stored covered in the fridge to a maximum of 7 days, If the Reactivation plate (MAX plate) is stored, DMSO needs to be replaced every day. (*See protocol for detailed protocol on plate preparation*)
4. When not performing an assay, do not leave the Reactivation plate on the Gator platform. It should always be stored at 4°C when not in use.
5. Bring the plate to room temperature before starting an assay every time it is brought out of 4°C
6. Assay setup is done using the "Flex K" option which has premade template for the reactivation plate. User may use regular "K" setup if altering the concentration of Flex SA Capture reagent and capture time.

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Kit Protocol

1. FLEX SA Kinetics protocol:

1.1 REACTIVATION PLATE SETUP

1.1.1 Bring Flex SA Capture Reagent (Sa-CR), Flex SA Reactivation Reagent, Flex SA Priming Reagent and K buffer to RT.
1.1.2 Aliquot out 2.5 ml of DMSO in a 5 ml tube.
1.1.3 Prepare the Max plate according to the following plate design.
1.1.4 Add 260 µL/well of Priming Reagent into columns 10 of a new Max Plate.
1.1.5 Add 300 µL/well of Flex SA Reactivation Reagent to column 1 of the Max plate.
1.1.6 Add 300 µL/well of 100% DMSO to column 2 of the Max plate.
1.1.7 Add 300 µL/well of Flex SA Priming Reagent to columns 3-5 and 7-9 of the Max plate.
1.1.8 Add 300 µL/well of Flex SA Capture reagent to column 6 of the Max plate.
**If desired, Flex SA Capture reagent may be diluted 1:2 or more in Flex SA Priming Reagent and loaded onto the probe for more than 2 minutes. See step 1.2.6*

The plate layout should be as shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Reactivation Reagent	DMSO 100%	Priming Reagent			Capture Reagent	Priming Reagent			Probe in Priming Reagent		
B												
C												
D												
E												
F												
G												
H												

1.1.9 Use the probe picker to pick out a column of fresh probes and place those in column 10, into which 260 µl wash buffer has been added in the previous step. Set the MAX plate aside and prepare the sample 96 well plate using the following design.

1.2 KINETIC ASSAY

1.2.1 Add 250 uL/well of K Buffer into columns 1, 3 and 5 of a black plate following the format below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	K buffer (Baseline)	Biotinylated sample (loading)	K buffer (Baseline)	Antibody (Associa-tion)	K buffer (Dissocia-tion)							
B												
C												
D												
E												
F												
G												
H												

1.2.2 Dilute biotinylated protein samples to desired concentrations using K buffer. Add samples to wells in column 2.
1.2.3 Prepare the antibody/analyte to desired concentrations using K buffer and add to column 4.
1.2.4 On the Gator, confirm Shaker A is in tilt position.
1.2.5 Place the black plate into Shaker A and the Max plate into Shaker B.
1.2.6 In the Gator software, start a new Flex K assay.

**If altering the concentration of Flex SA Capture Reagent (SA-CR), assay may be setup in "K" in order to optimize the loading time for SA-CR. The reactivation plate setup will have to be entered manually to match the one in "FLEX K" setup. SA-CR loading time will have to be optimized for every concentration. A loading time of 5 minutes is appropriate if SA-CR is diluted 1:2.*

1.2.7 Under Description, input required information.

1.2.8 Under Basic Parameters, input the following parameters:

- Data Acquisition: 5 Hz
- Shaker Setting: Tilt; A & B at 30°C
- Pre-wet & Pre-Mix Setting: 300 sec; Shaker A & B at 1000/1000 rpm

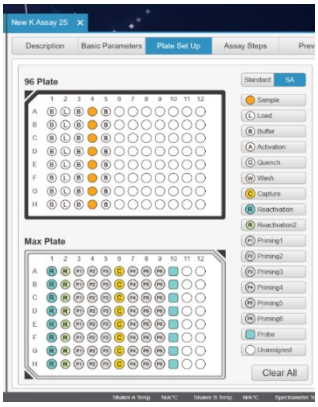
1.2.9 Under Plate Set Up, select SA and set up the assay plate maps. Highlight Columns 1, 3, and 5, in the 96 Plate and select the Buffer icon.

1.2.9.1 Highlight Column 2 in the 96 Plate and select the Load icon.

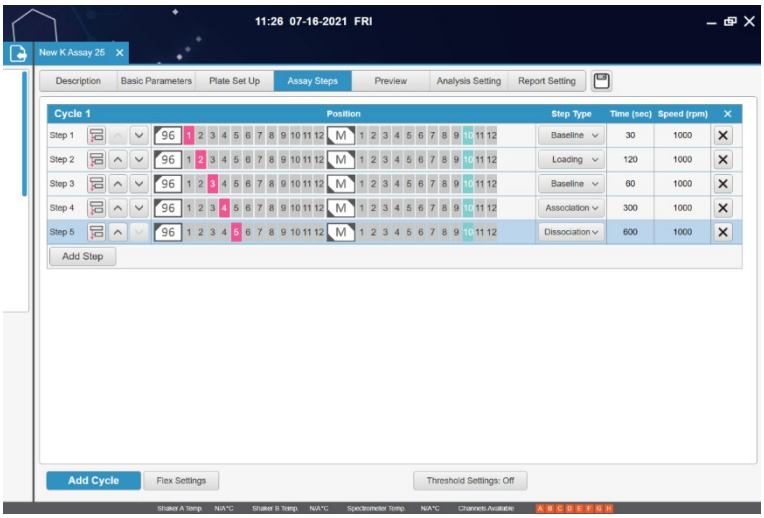
1.2.9.2 Highlight Column 4 in the 96 Plate and select the Sample icon.

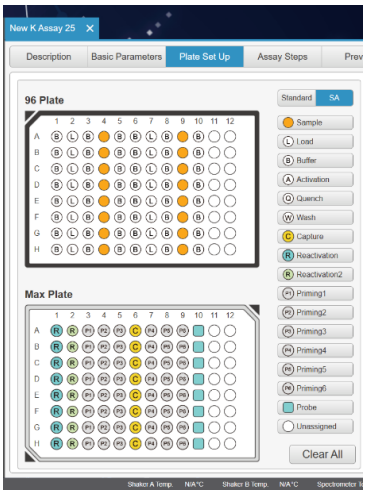
1.2.9.3 Specify molar concentrations for each sample well.

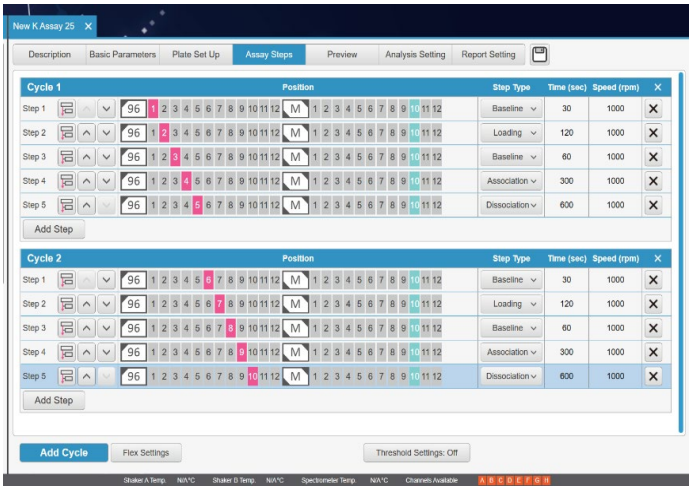
1.2.9.4 Highlight Column 10 in the Max Plate and select the Probe icon.



1.2.10 Under Assay Steps, set up the assay cycle(s) as follows:







1.2.11 If regenerating the probes to test another sample, hit "add cycle" and setup the baseline, loading, association, and dissociation wells.

Note: Make sure the appropriate wells are selected under "Plate Setup" before setting up in "assay steps". An example is shown below:

Note: If "K" setup is used instead of "Flex K", The assay steps for reactivation plate need to be added as shown below:

Note: For regeneration of probes, if "K" setup is used instead of "Flex K", click "Regeneration Settings" under Assay steps. Turn both "Regeneration" and "Regeneration before assay "On"

1.2.12 Under Preview, check if all the steps are correct and start the assay.

1.2.12 After assay is complete, required data analysis can be performed.