gator

Product Insert

Amine Reactive Probes

Catalog No. 160008

OVERVIEW

Gator™ Amine Reactive (AR) Probes are useful for determining kinetics of molecular interactions between protein and analyte. The AR probe surface comes precoated for the covalent attachment of a purified protein. The coupling process occurs through an EDC-activated amide bond between the reactive amine on the protein and the carboxy terminated probe surface. AR probes are well suited for high-affinity interactions due to the covalent immobilization.

MATERIALS REQUIRED

Amine Reactive Probes	Catalog No. 160008
Max Plate	Catalog No. 130062
Black Plates	Greiner 655209
1-Ethyl-3-(-3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) stock in water at 400 mM, store at -20 °C	ThermoFisher Scientific 22981B
N-Hydroxysulfo-succinimide sodium salt (Sulfo-NHS or s-NHS) stock in water at 200 mM, store at -20 °C	Sigma-Aldrich 56485-1G
1 M Ethanolamine (ETA) pH 8.5	Sigma-Aldrich 411000
10 mM sodium acetate buffer at each pH needed for pH scouting tests (typically pH 4, 5 and 6)	Sigma-Aldrich S5889

STORAGE

Store 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.

GENERAL APPLICATIONS

1. Kinetic assays of protein-protein interactions
2. Indirect quantitation assays
3. Epitope binning

GENERAL METHODS

Sample Volume

Black Plate: 200 µL (180 µL minimum)
Max Plate: 250 µL (280 µL maximum)

Pre-wet Conditions

250 µL volume DI water Max Plate,
5 min at 1000 rpm

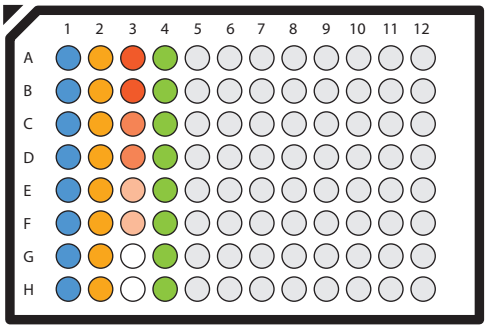
Speed

K	1000 rpm

General method for coupling

The optimal pH for covalent coupling to Gator AR probes will vary based on the protein being coupled. pH scouting should be performed to identify the optimal pH to be used for each protein. After diluting the protein of interest in acetate buffers of various pH, an experiment is performed in which the optimal pH will be indicated by the highest amount of protein coupled, as illustrated by the highest nm shift. *Reminder: purified proteins or peptides to be conjugated on AR probes must be in the buffer without amine (no Tris or glycine).*

Step Number	Step	Material	Instructions	Time (Sec)	Shake Speed (rpm)
1	Baseline	DI water	200 µL/well	60	1000
2	Activation	EDC/sNHS	Thaw EDC and sNHS stock. Mix 85 µL EDC and 85 µL sNHS in 1530 µL DI water to obtain concentrations of 20 mM EDC and 10 mM sNHS. Place 200 µL in each well. Mix and use immediately for best activity.	300	1000
3	Conjugation	Protein in acetate buffer of various pH	Dilute protein in acetate buffer to obtain concentrations of 5 to 20 µg/mL.	300	1000
4	Quench	ETA	200 µL/well	300	1000



DI water
EDC and Sulfo-NHS
protein in acetate buffer of various pH and control wells with no protein
ETA pH 8.5

Kinetic assays using AR probes

Once optimal pH has been determined for a protein, coupled AR probes can be used to measure high affinity protein-protein interactions in kinetic assays. The assay steps shown below represent an ideal recommended setup for an assay.

EXAMPLE ASSAY STEPS

Step Number	Step	Material	Time (Sec)	Shake Speed (rpm)
1	Baseline	DI water	60	1000
2	Activation	EDC/sNHS	300	1000
3	Conjugation	Protein in acetate buffer	120-300	1000
4	Quench	Ethanolamine	300	1000
5	Custom Baseline	K buffer	300	1000
6	Association	Analyte in K buffer	300-900	1000
7	Dissociation	K buffer	600-1800	1000

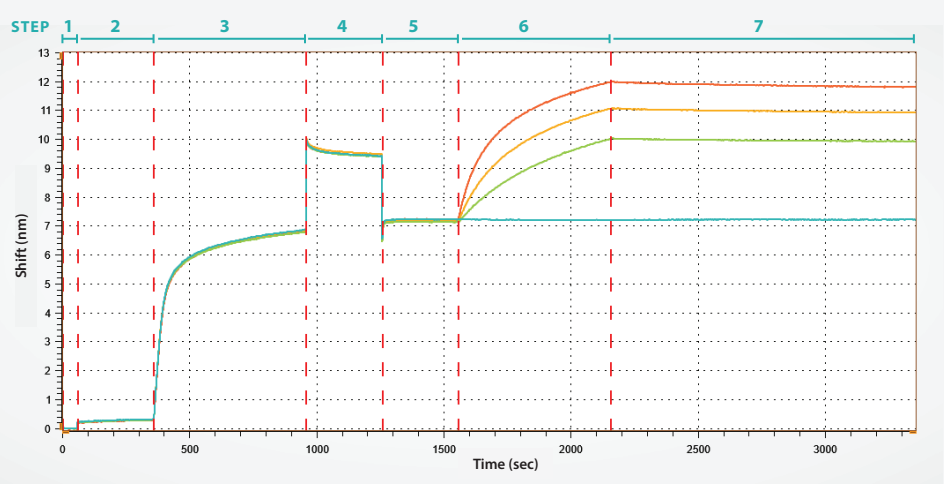


Figure 1: Amine Reactive Probe loaded with mouse IgG

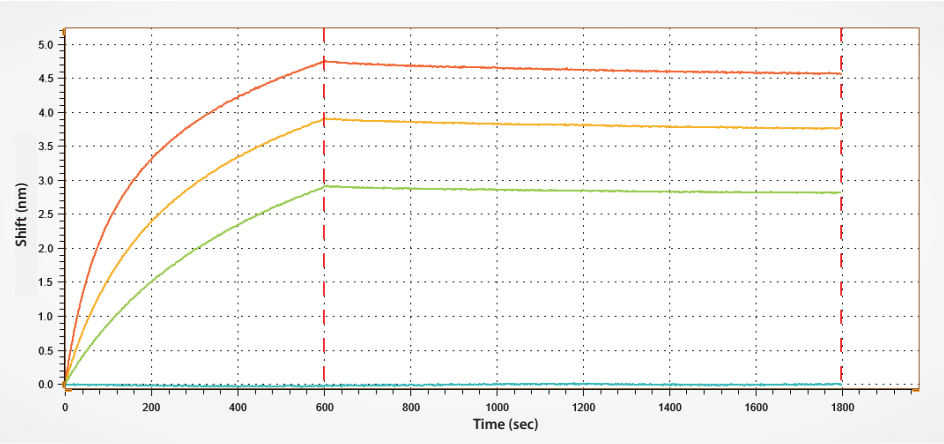


Figure 2: Association and dissociation kinetics of anti-mFc antibody= in a two-fold series dilution from 12.5 nM to 50 nM. 2.5 ug/mL mIgG was loaded.

k_{obs} (1/s)	4.04E-03
k_{off} (1/s)	2.66E-05
k_{on} (1/M*s)	1.37E+05
K_D (nM)	1.93E-10