Gator ™ Label-Free Analysis

Picomolar (pM) Buffer

PRODUCT DESCRIPTION

BUFFER: pM Buffer   **PACKAGE:** 1 x 50 mL   **PART NUMBER:** 120044

STORAGE: The use-before date applies to buffer stored at 2°C to 8°C in unopened containers.

SAFETY: For safe use and handling of the product, please refer to the Safety Data Sheet.

NOTE: For in vitro and research use only.

INTENDED USE

pM buffer can be used as the assay diluent for kinetics analysis using the Gator system. pM buffer is most suited for kinetics analysis of tight binding pairs in the nM to pM range, and it is compatible with SA Probe and AR Probe.

EXAMPLES OF USE

For the best result, pM Buffer should be used as the diluent in all steps of the kinetics assay. Do not dilute the buffer prior to use.

Kinetics analysis of several popular tight binding antibody drug pairs were tested using pM Buffer as diluent buffer. The resulting affinity values are all similar to published Biacore data. These binding pairs are: TNF-α and Adalimaub (Gator K_D: 20 pM, Biacore 30 pM); RBD and anti-SARS-CoV-2 antibody (Gator K_D: 24 pM, Biacore: N/A); VEGF and anti-VEGFa antibody (Gator K_D: 86 pM, Biacore 22 pM) and CRP and anti-CRP C2 antibody (Gator K_D: 8.6 pM, Biacore 2 pM).

TNFα and Adalimaub (anti-TNFα)

Step	Well Contents	Time (sec)	Speed (rpm)
Pre-Wet	pM Buffer, 200 µL / well	300	1000
Baseline	pM Buffer, 200 µL / well	60	1000
Loading	5 ug/mL Biotinylated-TNFα in pM Buffer, 200 µL / well	300	1000
Baseline	pM Buffer, 200 µL / well	300	1000
Association	0- 200 nM Adalimaub in pM Buffer, 200 µL / well	600	1000
Dissociation	pM Buffer, 200 µL / well	1800	1000
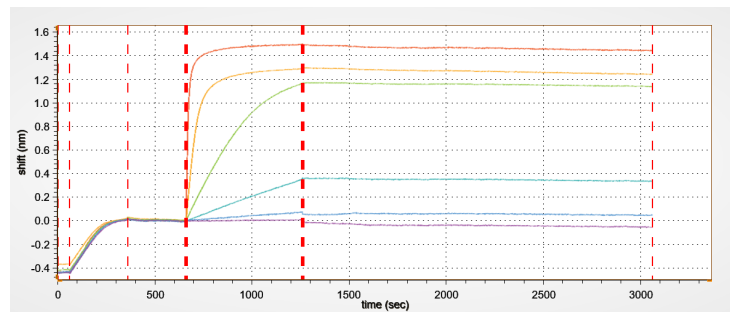
Published Biacore K_D: 60pM

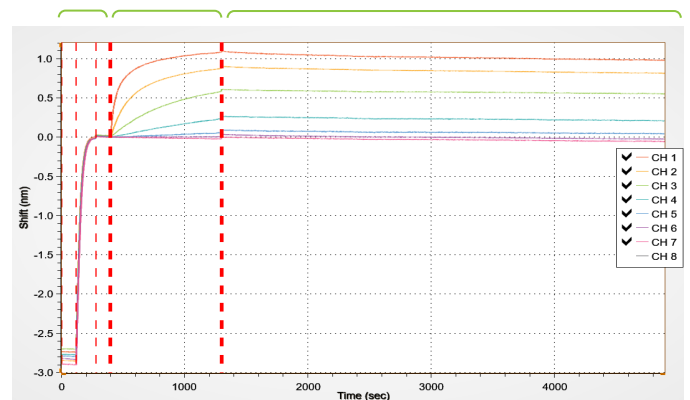Data Analysis: B-TNFα and anti-TNFα using pM Buffer

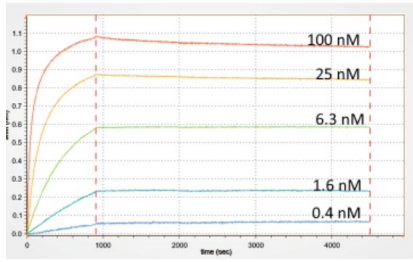| Fitting Method	Global Rmax Unlinked
R^2	0.9992
X^2	5.788
K_{off}	5.88 E -6 (1/s)
K_{on}	2.99 E D (1/Ms)
K_D	1.96 E – 11 (M)
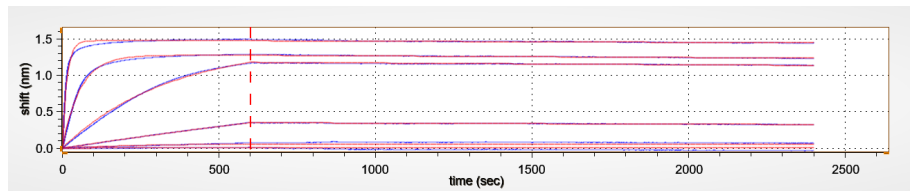
RBD and anti-SARS-CoV-2 antibody

The interaction between Biotinylated-RBD and a monoclonal anti-SARS-CoV-2 IgM antibody (Absolute Antibody#CR3022) was measured using pM Buffer.
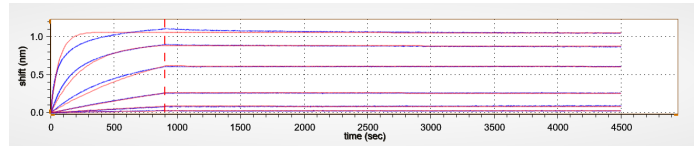
Step	Well Contents	Time (sec)	Speed (rpm)
Pre-Wet	200 µL / well	300	1000
Baseline	200 µL / well	120	1000
Loading	5 µg/mL Biotinylated-RBD in pM Buffer, 200 µL / well	158	1000
Baseline	PM Buffer, 200 µL / well	120	1000
Association	0- 200 nM anti-SARS-CoV2 antibodies in pM Buffer, 200 µL / well	900	1000
Dissociation	pM Buffer, 200 µL well	3600	1000

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Data Analysis: B-RBD and anti-RBD (Absolute Antibody#CR3022) using pM Buffer

| Fitting Method	Global Rmax Unlinked
R^2	0.9974
X^2	6.324
K_{off}	3.90 E -6 (1/s)
K_{on}	1.63 E +5 (1/Ms)
K_D	2.4 E -11 M (24 pM)

Data Analysis: B-RBD and anti-RBD (Sino#40592-T62) using pM Buffer

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VegF and anti-VegF (Biacore expected: 25 pM)

Step	Well Contents	Time (sec)	Speed (rpm)
Pre-Wet	200 µL / well	600	1000
Baseline	200 µL / well	120	1000
Loading	5 µg/mL Biotinylated-VegF (Sino PN: 11066-HNAH) in pM Buffer, 200 µL / well	251	1000
Baseline	pM Buffer, 200 µL / well	260	1000
Association	0- 100 nM Bevacizumab (anti-VegF) in pM Buffer, 200 µL / well	600	1000
Dissociation	pM Buffer, 200 µL well	3600	1000
Baseline	pM Buffer, 300 µL / well	3600	1000
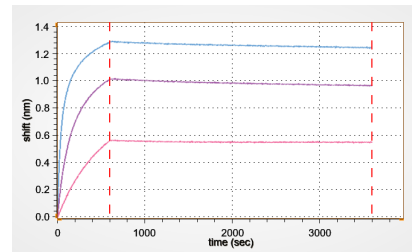
Fitting Method	Global Rmax Unlinked
R^2	0.9998
X^2	2.201
K_{off}	6.85 E -6 (1/s)
K_{on}	7.95 E 4 (1/Ms)
K_D	8.6 E – 11 (M), 86 pM
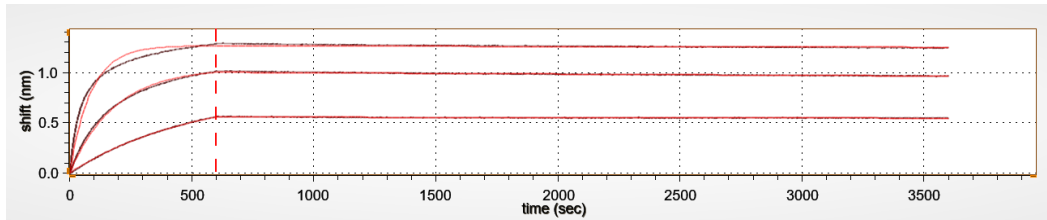
Data Analysis: B-VEGFa and anti-VEGFa using pM Buffer