Gator® Anti-VHH Probes

PRODUCT INSERT
Part Number: 160032

Overview

Gator® Anti-VHH probes are coated with Anti-VHH antibody cocktail to capture camelid VHHs (VHH: **V**ariable **H**eavy domain of **H**eavy chain) from different species.

VHHs are the variable domains of heavy-chain-only antibodies, with many unique properties such as small size (~15kDa), excellent solubility, superior stability, ease of manufacture, quick clearance from blood, and deep tissue penetration Researchers are exploring the potential of VHHs in the development of new treatments for various diseases and as diagnostic tools. The Anti-VHH probe can be used for quantitation of camelid VHHs (VHH) from different species and for off-rate screening.

Product Information

Materials Required

- Anti-VHH Probe, PN: 160032
- Quantitation (Q) buffer, PN: 120010
- Kinetics (K) buffer, PN: 120011
- Regen buffer (No Salt), PN: 120063
- Gator Max Plate, PN: 130062
- Gator BLI 96-Flat Plate, PN: 130260
- Precision Pipettes, User Supplied
- Sterile Pipette Tips, User Supplied

Storage Conditions

Store the tray in its foil packaging pouch at room temperature (RT), ensuring that the zipper is fully sealed. Probes are stable at RT for 1 year.

General Methods

Sample Volume

Max Plate: 250 - 280 µL/well
96-well Black Plate: 180 µL - 200 µL/well
384-well Black Plate: 80 µL - 100 µL/well

Prewet Conditions

250 µL/well in Max Plate for 10 minutes at 1000 rpm in Q buffer.

Regeneration Conditions

1000 rpm for 5 sec each in Regen and neutralization buffers (buffer diluent) for total of three cycles.
Note: *Regenerate fresh probes before the first assay to ensure consistency in data*.

Quantitation in Buffer

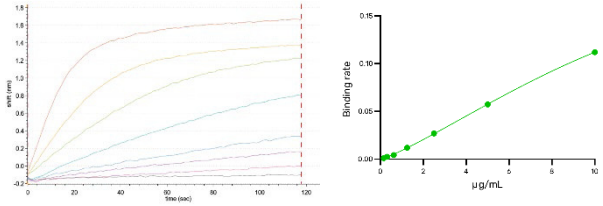

Fig. 1. Sensorgram and standard curve for VHH (0.156 to 10 µg/mL in Q buffer). Assay performed at 200 rpm for 120 sec.

Conc. (µg/mL)	Binding Rate	Binding rate %CV (n=4)	Calculated Conc. (µg/mL)	Calculated conc %CV (n=4)
10	0.112	0.85%	9.993	0.13%
5	0.0551	1.85%	4.995	2.59%
2.5	0.0272	2.38%	2.505	1.04%
1.25	0.0119	0.98%	1.268	1.41%
0.625	0.00438	1.59%	0.572	1.63%
0.313	0.00253	4.12%	0.365	1.95%
0.156	0.00115	1.80%	0.160	5.00%

Table 1: Accuracy and precision of VHH quantitation using standard protocol. Concentrations were calculated using initial binding rate.

High Sensitivity Quantitation in Buffer

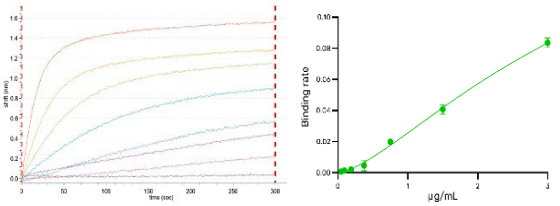

Fig. 2. Sensorgram and standard curve for VHH (0.05 to 3 µg/mL in Q buffer). Assay performed at 1000 rpm for 300 sec.

Conc. (µg/mL)	Binding Rate	Binding rate %CV (n=4)	Calculated Conc. (µg/mL)	Calculated conc %CV (n=4)
3	0.0804	1.03%	3.035	1.11%
1.5	0.0378	2.61%	1.463	3.22%
0.75	0.0189	3.79%	0.763	3.13%
0.375	0.0085	1.68%	0.404	2.92%
0.188	0.00206	3.09%	0.174	4.48%
0.094	0.0014	9.63%	0.098	9.20%
0.047	0.000786	12.11%	0.050	11.22%

Table 2: Accuracy and precision of VHH quantitation using high sensitivity protocol. Concentrations calculated using initial binding rate.

Regeneration

Anti-VHH probes can be regenerated up to 10 times using Gator's Regen buffer. Regeneration before assay is required to ensure run-to-run consistency.

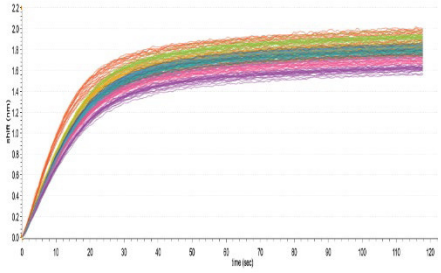

Fig. 3. 10 consecutive measurements of a VHH (10 µg/mL in Q Buffer) on 8 Anti-VHH probes with regeneration. Assay performed using standard protocol (200 rpm for 120 sec). % CV of concentrations for each probe is less than 1.5%.

Quantitation in Culture Media

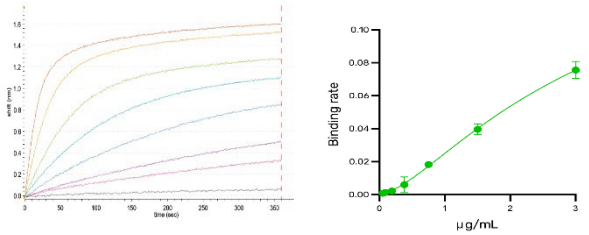

Fig. 4. Sensorgram and standard curve for 0.05 to 3 µg/mL VHH in spent DMEM media diluted 100 x in Q buffer. Similar results were seen with spent 2x YT and spent CHO media. Assay performed using high sensitivity protocol (1000 rpm for 300 sec).

Off-Rate Analysis

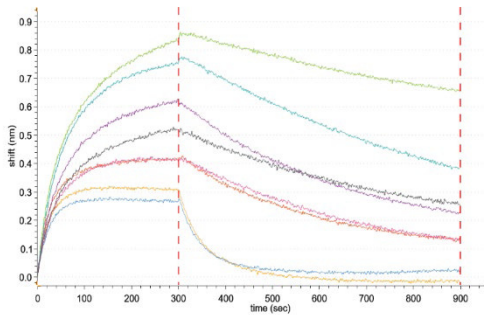

Fig. 5. Association and dissociation of eight VHHs against CD45. Eight different Anti-CD45 VHHs were loaded at 1 µg/mL in K Buffer onto Anti-VHH probes. The baseline after VHH loading was run for 10 minutes. CD45 was then loaded at 4 µg/mL in K buffer, followed by dissociation. The k_{off} values ranged from 3.9×10^{-4} to 1.48×10^{-4}.

Note: >10 minute baseline after VHH loading is recommended,

Q assay Issues

Question	Suggested Solution
Does the VHH probe bind to all types of nanobodies?	The VHH probe shows binding activity to all types of camelid nanobodies, lama, alpaca etc.
How should I quantitate samples with low binding?	For quantitation use the long protocol with 1000rpm
Lag phase with high concentration of samples	In the analysis remove first 2sec of the assay to fit the curve
What fitting model should be use for Q assay analysis?	InitialSlopeOptimal fitting equation
How should non-specific binding with the reference/media be eliminated?	Pre-wet the biosensors with the reference/media for 15minutes
Does the probe cross-react with other molecules?	VHH probes only show mild cross-reactivity to hIgG1, IgG3 and IgG4
Should the sensor be regenerated before an assay?	VHH probes needs to be normalized by regenerating before assay
Can I use undiluted media?	The VHH biosensors shows strong NSB to any undiluted media, it is suggested to dilute the media in PBST, Q or K buffer
What type of buffer is ideal for the experiment?	Any buffer that has a surfactant or surfactant and a blocking agent together is needed for the experiments. The VHH tends to adhere to the plate walls if the buffer is not optimal

K assay Issues

Question	Suggested Solution
Can I used the VHH probes for K assay?	Yes
After loading, the following wash step VHH dissociation is very strong. What should I do?	For all kinetic experiments we recommend the wash step after loading the VHH should be 10 minutes minimum or optimized to your VHH
I do not see any binding to my analyte/antigen after loading the VHH?	VHH is a 15 kDa small fragment, sometimes the antigen binding epitopes are hidden. We always recommend reversing the assay format
I see a lot of non-specific binding with the analyte binding to the VHH probes without loading	Please use a buffer which has surfactant and BSA, pre-wet for a longer period of time
I am using PBS as the running buffer and I see VHH binding signal going down	The 96 or 384 well plates need surfactant and BSA to block the surface. If you do not have these reagents present, the VHH will slowly start adhering to the plate surfaces
Some VHH has faster off rate after loading some has slow off rate. Why is this?	The binding affinities for VHH varies to the anti-VHH antibodies on the probe.

