Gator® Anti-PEG Probe

PRODUCT INSERT
Part Number: 160034

Overview

Gator® Anti-PEG probes are coated with Anti-PEG antibodies to capture Polyethylene Glycol (PEG) conjugated lipid-based drug carriers with pM affinity.

PEGylation of lipids is commonly done to increase stability and prolong biological half-life of lipid-based drug carriers. It reduces toxicity and immunogenicity, while enhancing tissue penetration.

PEGylation has significantly contributed to the development of more effective and safer biopharmaceuticals, and it continues to be an area of active research in the fields of medicine and biotechnology.

The Anti-PEG probe can be used for quantitation and binding studies of PEGylated lipid-based molecules such as LNPs, liposomes and nanoparticles.

Product Information

Materials Required

- Gator Max Plate, PN: 130062
- Gator BLI 96-Flat Plate, PN: 130260
- PBS + 0.2% BSA buffer, User Supplied
- 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 PBS + 0.2% BSA.

*Note: Q Buffer (120010), K Buffer (PN 120011), and detergents other than CHAPS are not compatible with anti-PEG probe.

PEGylated LNPs: Quantitation in in Buffer

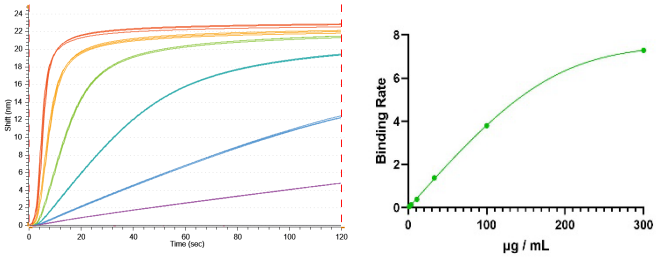

Fig. 1. Binding curves and standard curve for PEGylated lipid nanoparticles (1 µg/mL to 300 µg/mL in PBS + 0.2% BSA). Assay performed at 400 rpm. PEGylated LNPs were provided by a collaborator.

Conc. (µg/mL)	Binding Rate	%CV (n=3)	Calculated Conc. (µg/mL)	%CV (n=3)
300	7.29	1.77%	300.41	4.57%
100	3.80	2.75%	99.82	3.46%
33.33	1.38	0.48%	33.81	0.46%
11.11	0.38	0.74%	10.32	0.68%
3.70	0.13	1.36%	3.72	1.43%
1.24	0.05	2.18%	1.23	3.65%

Table 1: Accuracy and precision of PEGylated LNP quantitation using the Anti-PEG probe. Concentrations were calculated using initial binding rate. All CVs are within 10%.

PEGylated LNPs: Quantitation in Serum

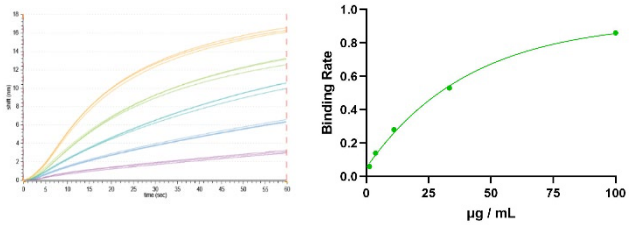

Fig. 2. Binding curves and standard curve for PEGylated LNPs (1.24 µg/mL to 100µg/mL in serum diluted 1:10 in PBS + 0.2% BSA). Probes were equilibrated in serum for 10 minutes before performing the assay at 400 rpm. PEGylated LNPs were provided by a collaborator.

Conc. (µg/mL)	Binding Rate	%CV (n=3)	Calculated Conc. (µg/mL)	%CV (n=3)
100	0.86	2.4%	100.13	6.22%
33.33	0.53	0.45%	33.33	0.88%
11.11	0.28	3.46%	11.13	5.57%
3.70	0.14	1.94%	3.70	2.74%
1.24	0.06	5.93%	1.24	7.17%

Table 2: Accuracy and precision of quantifying PEGylated LNPs in serum using the Anti-PEG probe. Concentrations were calculated using initial binding rate. All CVs are within 10%.

PEGylated Liposome Quantitation

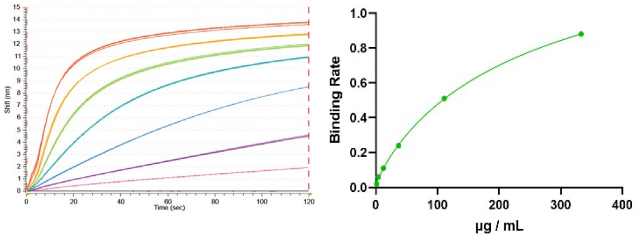

Fig. 3. Binding curves and standard curve for PEGylated liposomes (1 µg/mL to 300 µg/mL in PBS + 0.2 BSA). Assay performed at 400 rpm. PEGylated Liposomes were purchased from Creative Biostructure.

Conc. (µg/mL)	Binding Rate	%CV (n=3)	Calculated Conc. (µg/mL)	%CV (n=3)
333.33	0.88	0.87%	333.21	2.13%
111.11	0.51	2.15%	111.49	3.63%
37.04	0.24	0.82%	36.73	1.15%
12.35	0.11	1.07%	12.11	1.42%
4.12	0.06	2.08%	4.90	3.00%
1.37	0.02	1.03%	1.37	1.72%

Table 3: Accuracy and precision of quantifying PEGylated liposomes using the Anti-PEG probe. Concentrations were calculated using initial binding rate. All CVs are within 10%.

PEGylated LNP Binding to Apolipoprotein E

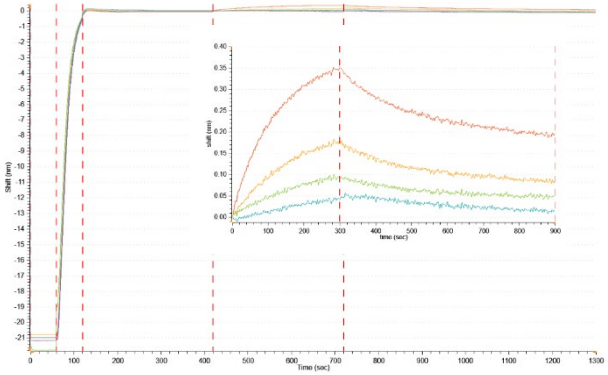

Fig. 4. Binding curves between PEGylated LNP and Apo-E, a protein involved in lipid transport. LNP was loaded at 20 µg/mL. Sensorgram shows association between 2.5, 5, 10 and 20 nM of Apo-E for 300 sec followed by dissociation for 600 sec. K_D = 2.93 nM. A positive signal is obtained for both LNP loading and subsequent Apo-E binding, unlike traditional BLI where inverted signals are often observed with lipid-based drug carriers.

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