

Research Use Only. Not for use in diagnostic procedures.

## Anti-6xHis AlphaLISA Acceptor Beads

**Product No.:** AL178C (250 µg)  
AL178M (5 mg)  
AL178R (25 mg)

### Product Formats

Catalog #	Size	Volume	Assay Points*
AL178C	250 µg	50 µL	500
AL178M	5 mg	1 mL	10 000
AL178R	25 mg	5 mL	50 000

\* The number of assay points is based on an assay volume of 25 µL using a final bead concentration of 20 µg/mL in 384-well format.

### Product Information

- Description:** Anti-6xHis AlphaLISA Acceptor beads at 5 mg/mL in PBS pH 7.2 with 0.05% Kathon as a preservative. The antibody used is a mouse monoclonal antibody.
- Application:** This product is intended for use in homogeneous AlphaLISA assays to capture 6xHis-tagged targets. Tris buffer is not recommended as an assay buffer since it affects product performance in the titration assay. Alpha Donor beads must be ordered separately.
- Storage:** Store in the dark at 4°C.
- Stability:** This product is stable for at least 12 months from the manufacturing date when stored in its original packaging under recommended storage conditions.

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### Quality Control

Lot-to-lot consistency is confirmed by a Quality Control AlphaLISA titration assay read on an EnVision® HTS Alpha instrument (see protocol below). We certify that the results meet our quality release criteria. *Note: maximum counts will vary depending on assay conditions as well as between lots. This variation has no impact on assay quality.*

## Titration Assay (Quality Control Protocol)

This protocol provides a means to verify product performance. It is used as our Quality Control release test. The following reagents and materials are used:

Item	Suggested Source	Catalog #
Glutathione Alpha Donor beads	PerkinElmer	6765300 (1 mg) 6765301 (5 mg) 6765302 (25 mg)
GST, 6 His-tagged	Millipore Corp.	12-523
AlphaLISA Universal Assay Buffer, 5X	PerkinElmer	AL001C (10 mL) AL001F (100 mL)
White OptiPlate™-384	PerkinElmer	6007290
TopSeal™-A Adhesive Sealing Film	PerkinElmer	6050195
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer	-

## Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to prewet the tip.
- Alpha Donor beads are light-sensitive. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Sodium azide should not be added to stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.
- Centrifuge the tubes briefly before use to improve recovery of content (2,000 x g, 10-15 sec). Resuspend all reagents by vortexing before use.
- Use Milli-Q® grade water (18 MΩ•cm) to dilute the 5X AlphaLISA Universal Assay Buffer.
- When diluting the probe, change tips after each dilution. When loading reagents in the assay microplate, change tips after each reagent addition and between each set of reagents.
- When reagents are added in the microplate, make sure the liquids are at the bottom of the well.
- 1X AlphaLISA Universal Assay Buffer contains PBS, pH 7.5, 0.1% BSA, 0.01% Kathon. This buffer is used in the titration assay described below (Quality Control Protocol). Optimization of the assay buffer might be necessary in other assay types.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Film to reduce evaporation during incubation. Microplates are read with the TopSeal-A Film on the plate.
- Total signal varies with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for all plates.

- The AlphaLISA signal is detected with an Alpha-enabled EnSpire or EnVision Multilabel Reader using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm, Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).

## Quality Control Protocol

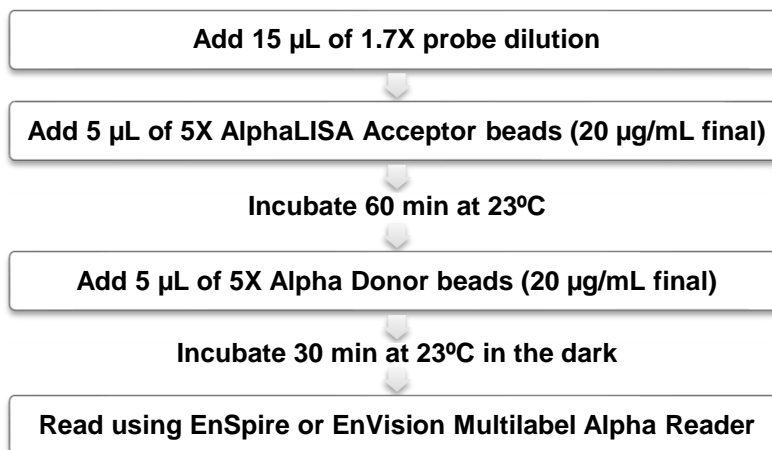
This titration protocol is designed for 12 dilutions of the probe with triplicate determinations. Final concentration of AlphaLISA Acceptor and Alpha Donor beads in the 25  $\mu\text{L}$  final assay volume is 20  $\mu\text{g}/\text{mL}$ . Volume of diluted reagents should be adjusted according to total number of assay points, plate format or assay volume.

- Preparation of 1X AlphaLISA Universal Assay Buffer:  
Add 1 mL of 5X AlphaLISA Universal Assay Buffer to 4 mL  $\text{H}_2\text{O}$ .
- Preparation of 1.7X probe (GST, 6 His-tagged) dilutions:  
Dilute probe to a 37  $\mu\text{M}$  stock solution.  
Prepare dilution series in 1X AlphaLISA Universal Assay Buffer as follows, changing tip for each dilution:

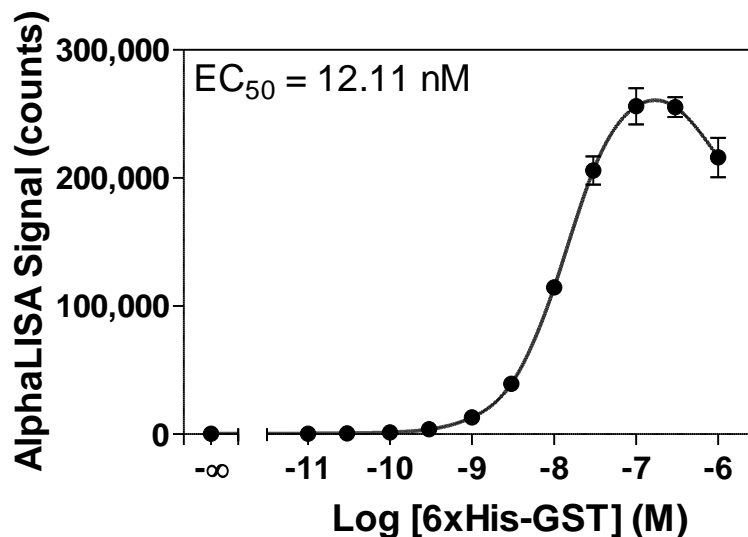
Tube	Volume of Probe	Volume of 1X Buffer ( $\mu\text{L}$ )	[GST, 6 His-tagged] (M)	
			in 15 $\mu\text{L}$ (1.7X)	in 25 $\mu\text{L}$ Final Assay Volume
A	8 $\mu\text{L}$ of 37 $\mu\text{M}$	166	1.7E-06	1.0E-06
B	60 $\mu\text{L}$ of tube A	140	5.1E-07	3.0E-07
C	60 $\mu\text{L}$ of tube B	120	1.7E-07	1.0E-07
D	60 $\mu\text{L}$ of tube C	140	5.1E-08	3.0E-08
E	60 $\mu\text{L}$ of tube D	120	1.7E-08	1.0E-08
F	60 $\mu\text{L}$ of tube E	140	5.1E-09	3.0E-09
G	60 $\mu\text{L}$ of tube F	120	1.7E-09	1.0E-09
H	60 $\mu\text{L}$ of tube G	140	5.1E-10	3.0E-10
I	60 $\mu\text{L}$ of tube H	120	1.7E-10	1.0E-10
J	60 $\mu\text{L}$ of tube I	140	5.1E-11	3.0E-11
K	60 $\mu\text{L}$ of tube J	120	1.7E-11	1.0E-11
L	0	140	0	0

- Preparation of 5X AlphaLISA Acceptor beads (100  $\mu\text{g}/\text{mL}$ ):  
Add 5  $\mu\text{L}$  of 5 mg/mL AlphaLISA Acceptor beads to 245  $\mu\text{L}$  of 1X AlphaLISA Universal Assay Buffer.
- Preparation of 5X Alpha Donor beads (100  $\mu\text{g}/\text{mL}$ ):  
Keep the beads under subdued laboratory lighting.  
Add 5  $\mu\text{L}$  of 5 mg/mL Alpha Donor beads to 245  $\mu\text{L}$  of 1X AlphaLISA Universal Assay Buffer.

5) In a white opaque OptiPlate-384 microplate:



## Typical Product Data



Titration assay using the Quality Control protocol. Signal was detected with an EnVision Alpha instrument 2102.

Please visit our website for additional information on the AlphaLISA technology at [www.perkinelmer.com/AlphaTech](http://www.perkinelmer.com/AlphaTech).

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