

AlphaLISA®

## Anti-Human IgE Acceptor Beads

**Product number:** AL176C **Lot Number:** 3355896

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**Material provided:** Anti-human IgE Alpha Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon as a preservative.

**Product Format:** AL176C: 250 µg, 50 µL, 500 assay points  
AL176M: 5 mg, 1 mL, 10 000 assay points  
AL176R: 25 mg, 5 mL, 50 000 assay points

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

**Manufacturing date:** 10/15/2024 **Document version:** 1

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### Product Information

**Application:** This product is intended for use in homogenous Alpha assays to capture human IgE.

**Storage:** Store product in the dark at 4 °C.

**Stability:** This kit is stable for at least 6 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

### Quality Control

Lot to lot consistency is confirmed in an Alpha assay. Maximum and minimum signals and EC50 were measured on the EnVision Multilabel Plate Reader with Alpha option. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on assay quality

EC<sub>50</sub>: 0.49 nM  
Min counts: 316 counts  
Max counts: 117,631 counts

### Titration Assay (Quality Control Procedure)

This protocol provides a means to verify product performance. The following reagents and materials are recommended.

Item	Suggested source
White OptiPlate™-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision®-Alpha Reader	Revvity Inc.
AlphaScreen® Streptavidin coated Donor Beads	Revvity Inc.
Biotin-human IgE	Revvity Inc.
AlphaLISA Immunoassay Buffer 10X	Revvity Inc.

## Recommendations

- AlphaScreen® 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) can be applied to light fixtures.
- Sodium azide should not be added to stock solutions or assay components. Final concentrations of sodium azide higher than 0.001 % will decrease the AlphaLISA signal.
- Spin down tubes briefly before use to improve recovery of content (2,000 x g, 10-15 sec). Resuspend all reagents by vortexing before use.
- 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 EnVision Multilabel Reader equipped with the ALPHA option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).

## Protocol

- Preparation of 1X AlphaLISA Immunoassay Buffer:  
Add 1 mL of 10X AlphaLISA Immunoassay Buffer to 9 mL H<sub>2</sub>O.
- Preparation probe dilutions:
  - Reconstitute the 2.35 µg lyophilized biotin hIgE in 50 µL Milli-Q H<sub>2</sub>O to make 250 nM stock solution.
  - Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

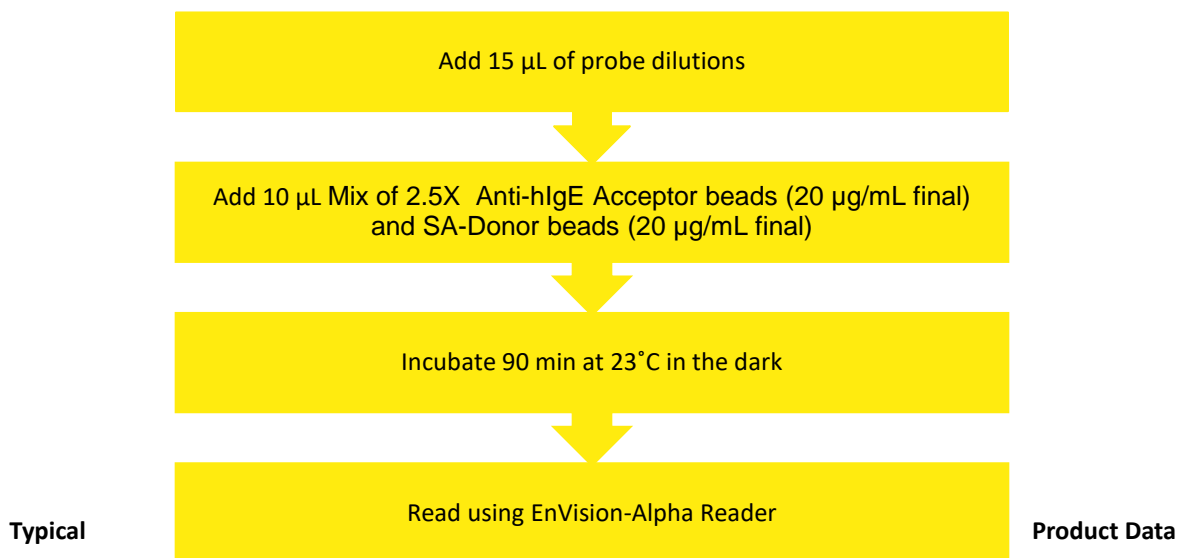
Tube	Volume of Probe	Volume of buffer (µL)	[hIgE] (M) (1.7X)	[hIgE] (pM)
A	10 µL of 250nM hIgE	140	1.7E-8	16,667
B	60 µL of tube A	140	5.0E-9	5,000
C	60 µL of tube B	120	1.7E-9	1,667
D	60 µL of tube C	140	5.0E-10	500

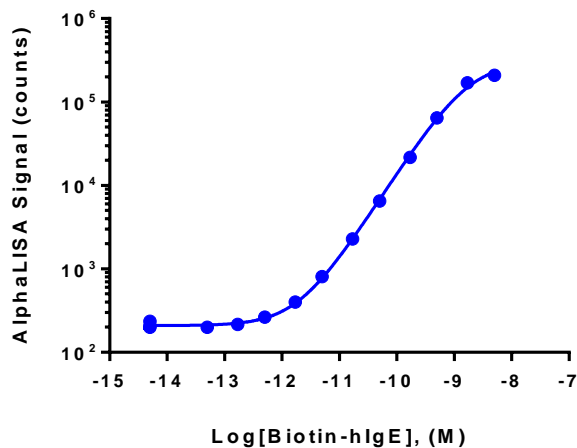
E	60 µL of tube D	120	1.7E-10	167
F	60 µL of tube E	140	5.0E-11	50
G	60 µL of tube F	120	1.7E-11	17
H	60 µL of tube G	140	5.0E-12	5
I	60 µL of tube H	120	1.7E-12	1.7
J	60 µL of tube I	140	5.0E-13	0.5
K	60 µL of tube J	120	1.7E-13	0.17
L	60 µL of tube K	140	5.0E-14	0.05
M	0	100	0	0
N	0	100	0	0
O	0	100	0	0
P	0	100	0	0

3) Preparation of the mix of 2.5X anti-hlgE Acceptor beads (50 µg/mL) and Streptavidin (SA) Donor beads (50 µg/mL) (50 µg/mL)

Add 10 µL of 5 mg/mL AlphaLISA anti-hlgE Acceptor beads and 10 µL of 5 mg/mL Streptavidin (SA) Donor beads into 980 µL of 1X AlphaLISA Immunoassay Buffer.

5) In a OptiPlate-384 microplate:





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

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