

Research Use Only. Not for use in diagnostic procedures.

Anti-Human IgG Acceptor Beads

Product No.: AL103C (250 µg)
AL103M (5 mg)
AL103R (25 mg)

Lot No.: 2613166

Material Provided

Formats:

| Catalog number | Size | Volume | Assay points |
|----------------|--------|--------|--------------|
| AL103C | 250 µg | 50 µL | 500 |
| AL103M | 5 mg | 1 mL | 10 000 |
| AL103R | 25 mg | 5 mL | 50 000 |

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:

August 16, 2019

Description:

AlphaLISA Anti-Human IgG Acceptor Beads at 5 mg/mL in PBS, pH 7.2 supplemented with 0.05% Proclin-300 as a preservative.

Storage:

Store in the dark at 4°C.

Stability:

This product is stable for at least 24 months from the manufacturing date when stored in its original packaging under recommended storage conditions.

Product Information

Intended use:

This product is intended for use in homogeneous AlphaLISA assays for the capture of human IgG. The anti-human antibody coupled to the beads targets the Fc region of human IgG.

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Quality Control

Lot-to-lot consistency is confirmed by a Quality Control AlphaScreen® 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.*

Maximum signal: 126,699 counts
Minimum signal: 632 counts
EC₅₀: 0.76 nM

Titration Assay (Quality Control Protocol)

This protocol provides a means to verify product performance.

The following reagents and materials are recommended.

| Item | Suggested Source | Catalog # |
|---|---|---|
| AlphaScreen® Streptavidin-coated Donor Beads | PerkinElmer LAS, Inc. | 6760002S (1 mg) 6760002 (5 mg) 6760002B (50 mg) |
| Biotin-human IgG | Jackson ImmunoResearch Laboratories, Inc. | 009-060-003 |
| AlphaLISA Universal Assay Buffer, 5X | PerkinElmer LAS, Inc. | AL001C (10 mL) AL001F (100 mL) |
| White OptiPlate™-384 | PerkinElmer LAS, Inc. | 6007290 |
| TopSeal™-A Adhesive Sealing Film | PerkinElmer LAS, Inc. | 6005185 |
| EnVision® Multilabel Reader with the Alpha Option | PerkinElmer LAS, 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 (preferred) or Roscolux filters #389 from Rosco) 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.
- Use Milli-Q® grade water (18 MΩ•cm) to dilute the 5X AlphaLISA Universal Buffer.
- 1X AlphaLISA Universal Assay Buffer contains PBS, pH 7.5, 0.1% BSA, 0.01% Proclin-300. 1X AlphaLISA Universal Assay Buffer is used in the titration assay described below (Quality Control Protocol). Optimization of this 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 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:

This protocol is recommended for generating one titration curve in a 25 μL final assay volume (12 concentrations, triplicate determinations with manual pipetting). If more assay points are needed, volumes should be adjusted accordingly.

1) Preparation of 1X AlphaLISA Universal Assay Buffer:

Add 1 mL of 5X AlphaLISA Universal Assay Buffer to 4 mL H_2O .

2) Preparation of 1.7X Biotin-human IgG dilutions:

Dilute Biotin-human IgG to a 50 nM stock solution.

Prepare 1.7X dilutions in 1X AlphaLISA Universal Assay Buffer as follows:

| Tube | Volume of Biotin-human IgG | Volume of buffer (μL) | [Biotin-human IgG] (M) in 15 μL (1.7X) | [Biotin-human IgG] (M) in final assay volume (25 μL) |
|------|----------------------------|------------------------------------|---|--|
| A | 51 μL of 50 nM | 99 | 1.7E-8 | 1.0E-8 |
| B | 60 μL of tube A | 140 | 5.1E-9 | 3.0E-9 |
| C | 60 μL of tube B | 120 | 1.7E-9 | 1.0E-9 |
| D | 60 μL of tube C | 140 | 5.1E-10 | 3.0E-10 |
| E | 60 μL of tube D | 120 | 1.7E-10 | 1.0E-10 |
| F | 60 μL of tube E | 140 | 5.1E-11 | 3.0E-11 |
| G | 60 μL of tube F | 120 | 1.7E-11 | 1.0E-11 |
| H | 60 μL of tube G | 140 | 5.1E-12 | 3.0E-12 |
| I | 60 μL of tube H | 120 | 1.7E-12 | 1.0E-12 |
| J | 60 μL of tube I | 140 | 5.1E-13 | 3.0E-13 |
| K | 60 μL of tube J | 120 | 1.7E-13 | 1.0E-13 |
| L | 0 | 120 | 0 | 0 |

3) Preparation of 5X AlphaLISA Acceptor beads (100 $\mu\text{g}/\text{mL}$):

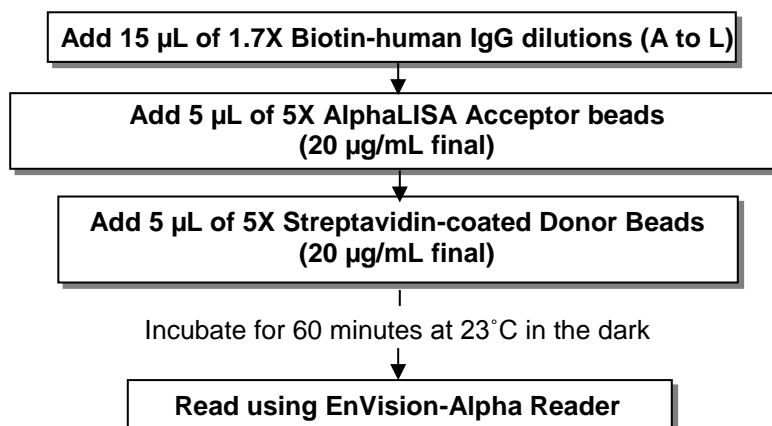
Add 5 μL of 5 mg/mL AlphaLISA beads to 245 μL of 1X AlphaLISA Universal Assay Buffer.

4) Preparation of 5X Streptavidin-coated Donor Beads (100 $\mu\text{g}/\text{mL}$):

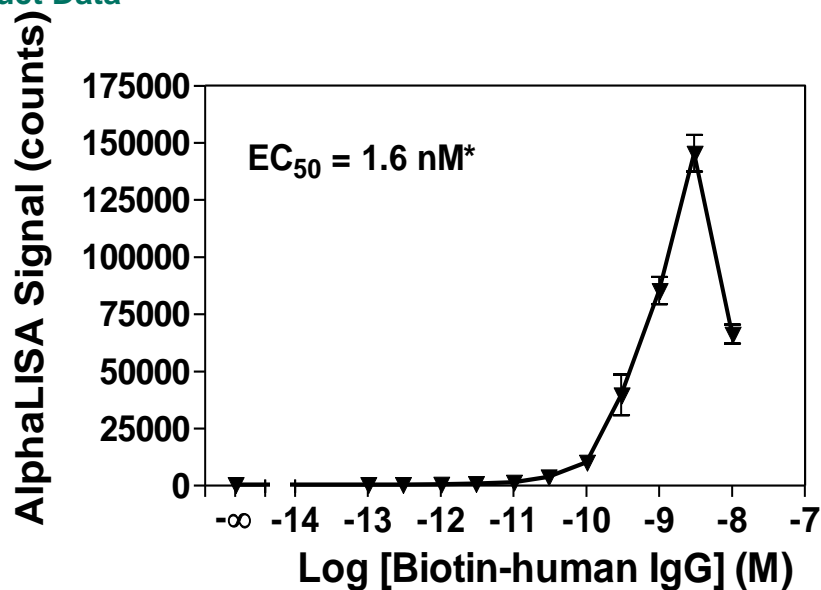
Keep the beads under subdued laboratory lighting.

Add 5 μL of 5 mg/mL Streptavidin-coated Donor Beads to 245 μL of 1X AlphaLISA Universal Assay Buffer.

5) In an OptiPlate-384 microplate:



Typical Product Data



* The EC₅₀ value was determined following a non-linear regression analysis using the sigmoidal dose-response curve model with variable slope. Only assay points up to the maximum signal were used for EC₅₀ determination (in this case, up to 3 nM).

Suggested Materials and Instrumentation

Please visit our website

www.perkinelmer.com/AlphaTech

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