CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaLISA®

Anti-Rat IgG Acceptor Beads

Product number:	AL106C	Lot Number:	3198071

Material provided: AlphaLISA Anti-Rat IgG Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon as a preservative.

Product Format: AL106C: 250 µg, 50 µL, 500 assay points

AL106M: 5 mg, 1 mL, 10 000 assay points

AL106R: 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:	09/12/23	Document version:	1

Product Information

Application:	This product is intended for use in homogeneous AlphaLISA assays for the capture of rat IgG. The anti-rat antibody coupled to the beads targets the Fc region of rat IgG.
Storage:	Store product in the dark at 4 °C.
Stability:	This kit is stable for at least 12 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_{50}:	0.66 nM
Min counts:	263 counts
Max counts:	99892 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-rat IgG	Jackson Immuno Research (CAT#012-060-003)
AlphaLISA Universal Assay Buffer 5X	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.
- 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% Kathon. 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

1) Preparation of 1X AlphaLISA Universal Buffer:
   Add 1 mL of 5X AlphaLISA Universal Buffer to 4 mL H_2O.

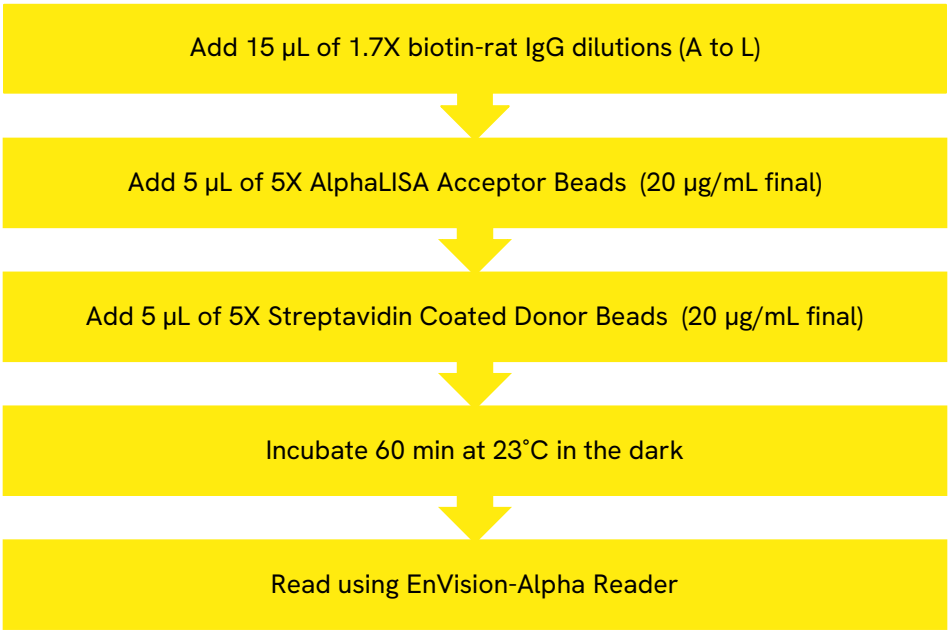
2) Preparation 1.7X Biotin-rat IgG dilutions:
   Dilute Biotin-rat IgG to a 50 nM stock solution
   Prepare 1.7X dilutions in 1X AlphaLISA Universal Assay Buffer as follows:

Tube	*Volume of* Biotin-rat IgG	Volume of buffer (µL)	[Biotin-rat IgG] (M) in 15 µL (1.7X)	[Biotin-rat IgG] (M) in final assay volume (25 µL)
A	51 µL of 50 µM	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	140	0	0

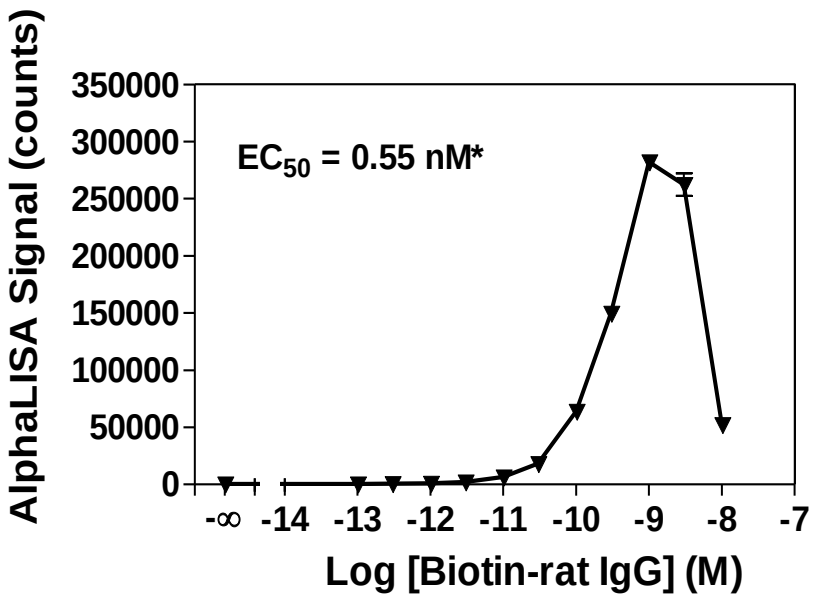
3) Preparation of 5X AlphaLISA Acceptor beads (100 µg/mL):)
Add 5 µL of 5 mg/mL AlphaLISA beads to 245 µL of 1X AlphaLISA Universal Assay Buffer.

4) Preparation of 5X Streptavidin-coated Donor Beads (100 µg/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 a 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 1 nM).

Please visit our website for additional information on AlphaLISA technology at www.revvity.com

AL106-R Rev01

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