CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaLISA®

Anti-HRP Acceptor Beads

Product number: AL171C **Lot Number:** 3330528

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

Product Format: AL171C: 250 µg, 50 µL, 500 assay points

AL171M: 5 mg, 1 mL, 10 000 assay points

AL171R: 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: August 15, 2024 **Document version:** 1

Product Information

Application: This product is designed for use as a tool to generate Alpha assays involving proteins such as antibodies coupled with the horseradish peroxidase (HRP) protein tag. This tag is quite commonly used in ELISA assays as a reporter for antibody binding and can be found on both primary and secondary antibodies. Direct ELISA conversion can be made easy with AlphaLISA beads binding directly to the HRP portion of ELISA's detection antibodies.

Storage: Store product in the dark at 4 ºC.

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}:	0.10 nM
Min counts:	360 counts
Max counts:	261924 counts

Titration Assay (Quality Control Procedure)

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

1) Preparation of 1X Immunoassay Buffer:

   Add 1mL of 10X Immunoassay Buffer to 9mL of MilliQ grade water.

2) Preparation probe dilutions:

   Prepare standard dilutions as follows in 1X Immunoassay Buffer (change tip between each standard dilution):

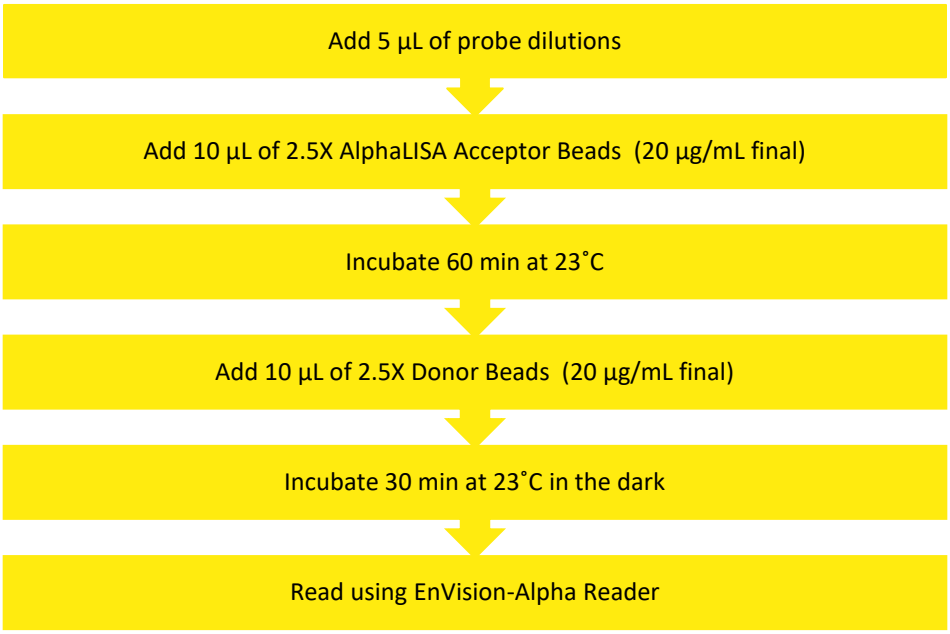
Tube	*Volume of* Probe	Volume of buffer (µL)	[Biotinylated ANTI-HRP] (M) (in 5 µL, 5X)	[Biotinylated ANTI-HRP] (M) (in 25 µL)
A	2.5 µL of 20 µM	97.5	5E-07	1E-07
B	30 µL of tube A	70	1.5E-07	3E-08
C	30 µL of tube B	60	5E-08	1E-08
D	30 µL of tube C	70	1.5E-08	3E-09

E	30 µL of tube D	60	5E-09	1E-09
F	30 µL of tube E	70	1.5E-09	3E-10
G	30 µL of tube F	60	5E-10	1E-10
H	30 µL of tube G	70	1.5E-10	3E-11
I	30 µL of tube H	60	5E-11	1E-11
J	30 µL of tube I	70	1.5E-11	3E-12
K	30 µL of tube J	60	5E-12	1E-12
L	0	100	0	0

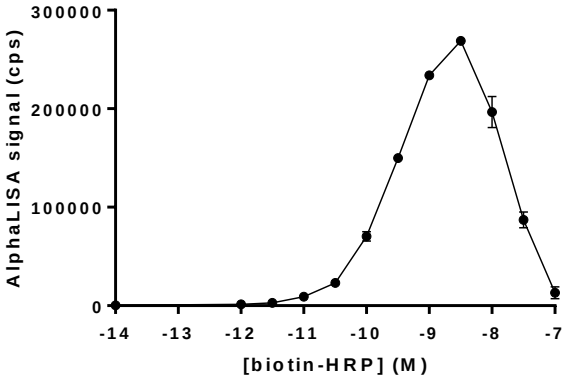
3) Preparation of 2.5X AlphaLISA Acceptor beads (50 µg/mL):
Add 15 µL of 5 mg/mL AlphaLISA Acceptor beads to 1485 µL of 1X Immunoassay Buffer

4) Preparation of 2.5X Streptavidin-coated Donor Beads (50 µg/mL):
Keep the beads under subdued laboratory lighting. Add 5 µL of 5 mg/mL Donor Beads to 495 µL of of 1X Immunoassay Buffer

5) In a OptiPlate-384 microplate:



Typical Product Data



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

AL171-R Rev01

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