CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaLISA®

C-tag Acceptor Beads

Product number: AL172C **Lot Number:** 3293186

Material provided: Anti-C-tag AlphaLISA Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Kathon as a preservative. The antibody utilized is an antibody that recognizes the C-tag protein tag sequence, coming from C-protein fusions (Sequence is biotin-EDQVDPRLIDGK). No significant cross reactivity was identified with other tags tested.

Product Format: AL172C: 250 µg, 50 µL, 500 assay points

AL172M: 5 mg, 1 mL, 10 000 assay points

AL172R: 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: May 30, 2024 **Document version:** 1

Product Information

Application: This product is intended for use in homogenous Alpha assays to capture C-tag labeled proteins. Alpha Donor beads must be ordered separately.

Storage: Store product in the dark at 4 ºC.

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_{50}:	3.23 nM
Min counts:	1963 counts
Max counts:	559314 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 Donor Beads	Revvity Inc.
Biotinulated C-tag (biotin-EDQVDPRLIDGK)	Anaspec

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 PBS + 0.1% Tween 20:
   Add 100 µL of 10% Tween 20 to 9.9 mL of PBS.

2) Preparation probe dilutions:

   a. Briefly vortex and briefly centrifuge (5 seconds) biotinylated Anti-C-tag (10 mM)
   b. Dilute sample to 100 µM by adding 1 µL to 99 µL of PBS + 0.1% Tween 20.
   c. Prepare standard dilutions as follows in 1X PBS + 0.1% Tween 20 (change tip between each standard dilution):

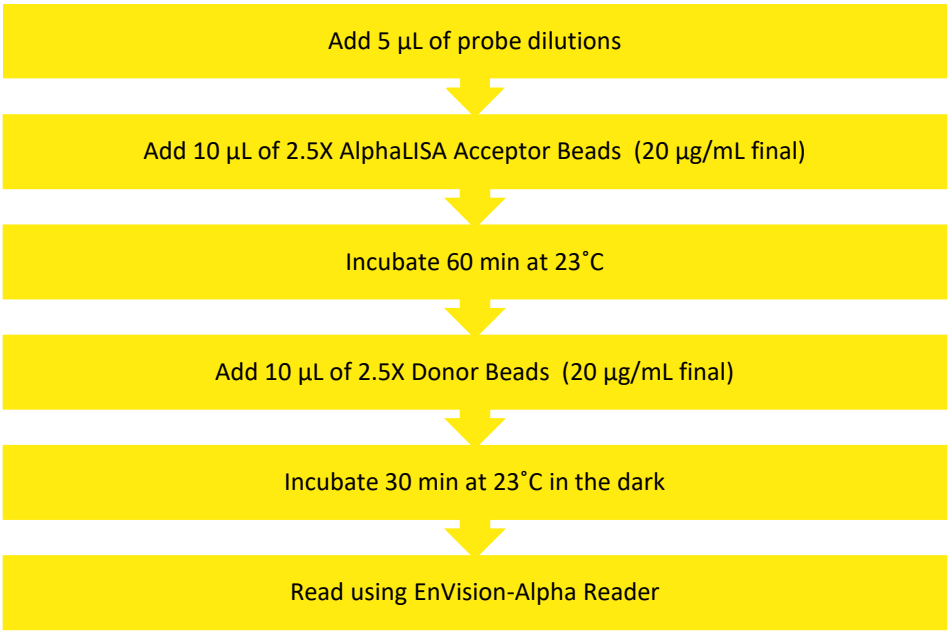
Tube	*Volume of* Probe	Volume of buffer (µL)	(M in 5 µL)
A	1 µL of 100 µM	99	1.0E-6
B	60 µL of tube A	140	3.0E-7
C	60 µL of tube B	120	1.0E-7

D	60 µL of tube C	140	3.0E-8
E	60 µL of tube D	120	1.0E-8
F	60 µL of tube E	140	3.0E-9
G	60 µL of tube F	120	1.0E-9
H	60 µL of tube G	140	3.0E-10
I	60 µL of tube H	120	1.0E-10
J	60 µL of tube I	140	3.0E-11
K	60 µL of tube J	120	1.0E-11
L	60 µL of tube K	70	3.0E-12
M	0	100	0

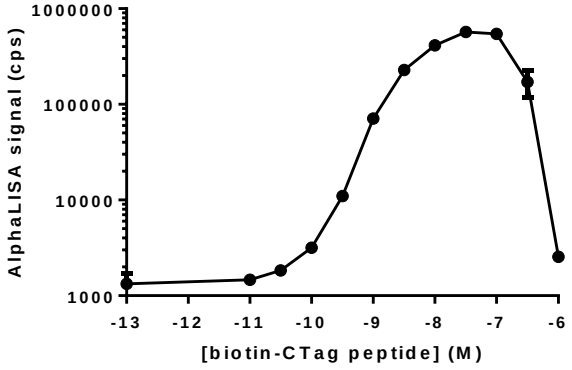
3) Preparation of 2.5X AlphaLISA Acceptor beads (50 µg/mL):
Add 20 µL of 5 mg/mL AlphaLISA Anti-C-tag Acceptor beads to 1980 µL of 1X PBS + 0.1% Tween 20.

4) Preparation of 2.5X Streptavidin-coated Donor Beads (50 µg/mL):
Keep the beads under subdued laboratory lighting. Add 20 µL of 5 mg/mL Streptavidin-coated Donor Beads to 1980 µL of of 1X PBS + 0.1% Tween 20.

5) In a OptiPlate-384 microplate:



Typical Product Data



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

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