TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

AlphaLISA C-tag Acceptor Beads

Product number: AL172 C/M/R

Lot number: 2549085

Manufacturing date: April 3, 2019

Research Use Only. Not for use in diagnostic procedures.

Contents

Product Information

Description: Anti-C-tag AlphaLISA Acceptor Beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Proclin-300 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.

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

Formats:

Catalog #	Size	Volume	Assay Points
AL172C	0.25 mg	50 µL	500
AL172M	5 mg	1000 µL	10 000
AL172R	25 mg	5000 µL	50 000

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

Storage: Store in the dark at 4°C.

Stability: This product is stable for at least 6 months from the manufacturing date when stored in its original packaging under recommended storage conditions.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum, minimum signals, and EC_{50} were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that the results meet our quality release criteria. Note: maximum counts will vary depending on assay conditions as well as between lots and instrument used. This variation has no impact on assay quality.

Maximum Counts: 505592 counts
Minimum Counts: 716 counts
$EC_{50:}$ 1.000 nM

Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute 10X PBS + 1% Tween-20.
- When diluting the probe, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Plus Film.
- 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, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.

Quality Control Protocol

This protocol provides a means to verify product performance. It is used as our Quality Control release test. The following reagents and materials are used in addition to the AlphaLISA Acceptor beads:

Item	Suggested Source	Catalog #
Streptavidin Alpha Donor Beads	PerkinElmer	6760001
Biotinylated C-tag (biotin-EDQVDPRLIDGK)	AnaSpec	Custom synthesis
White OptiPlate™-384	PerkinElmer	6007290
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer	6050185
PBS + 0.1% Tween 20	Custom	
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer	-

Assay Protocol

This titration protocol is designed for 12 dilutions of the probe with triplicate determinations. Final concentration of AlphaLISA Acceptor and Alpha Donor beads in the 25 µL final assay volume is 20 µg/mL. Volume of diluted reagents should be adjusted according to total number of assay points, plate format or assay volume.

Steps for Preparing Reagents

1) Preparation of 1X PBS + 0.1% Tween 20:
Add 100 µL of 10% Tween 20 to 9.9 mL of PBS.

2) Preparation of biotinylated C-tag standard 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	Vol. of Biotin-C-tag (µL)	Vol. of diluent (µL) *	(M in 5 µL)
A	1 µL of 100 µM	99	1.00E-06
B	60 µL of tube A	140	3.00E-07
C	60 µL of tube B	120	1.00E-07
D	60 µL of tube C	140	3.00E-08
E	60 µL of tube D	120	1.00E-08
F	60 µL of tube E	140	3.00E-09
G	60 µL of tube F	120	1.00E-09
H	60 µL of tube G	140	3.00E-10
I	60 µL of tube H	120	1.00E-10
J	60 µL of tube I	140	3.00E-11
K	60 µL of tube J	120	1.00E-11
L	60 µL of tube K	140	3.00E-12
M ** (background)	0	100	0

* Dilute standards in diluent (e.g. 1X PBS + 0.1% Tween 20).
At low concentrations, a significant amount of probe can bind to the vial. Therefore, load the probe dilutions into the assay microplate within 60 minutes of preparation.

** For calculating background signal one background point in triplicate can be used (3 wells).

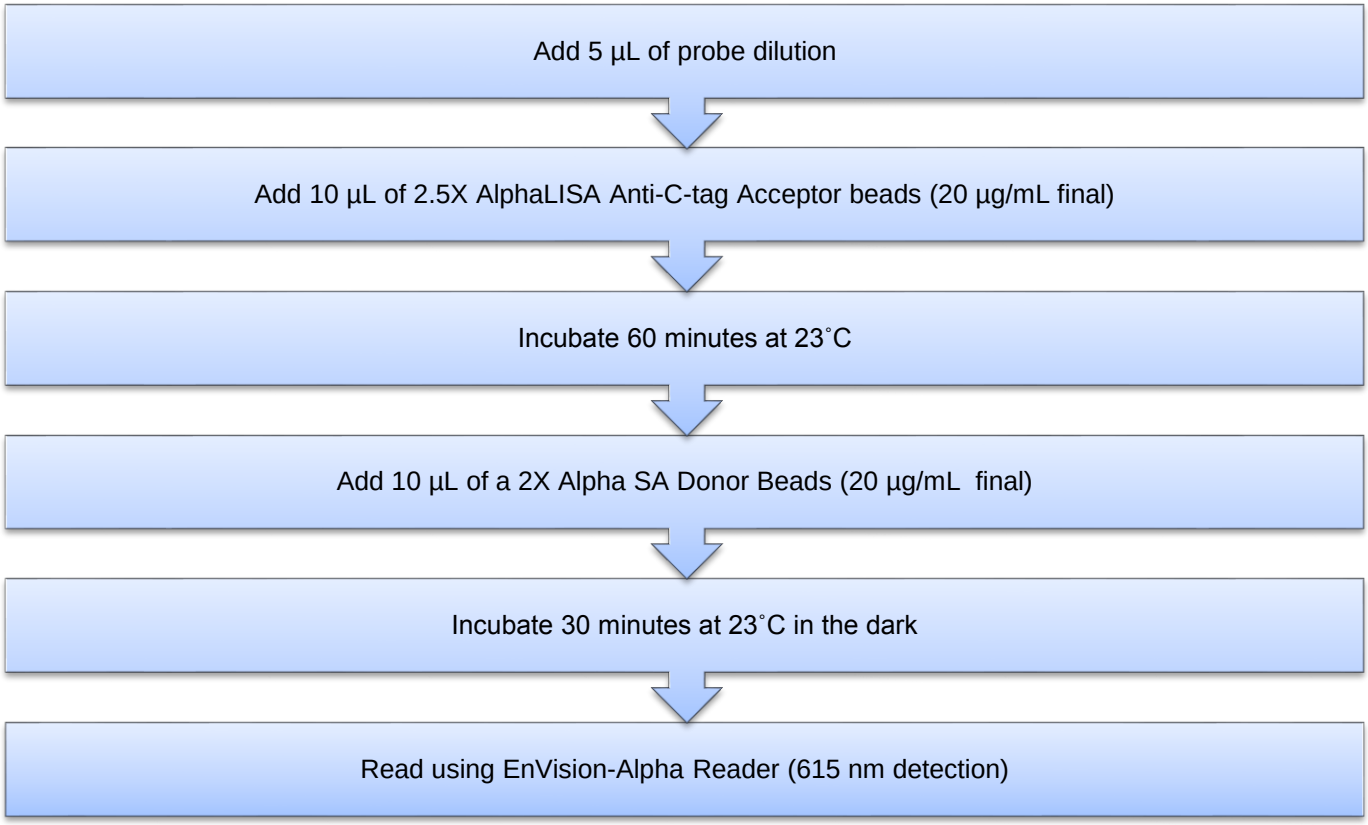
3) Preparation of 2.5X Alpha Anti-C-tag Acceptor beads (50 µg/mL)

a. Prepare just before use and keep the beads under subdued laboratory lighting.
b. 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 (SA) Donor beads (50 µg/mL):

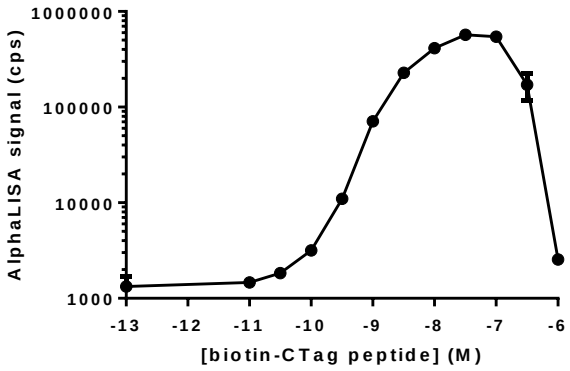
a. Prepare just before use.
b. Add 20 µL of 5 mg/mL SA Acceptor beads to 1980 µL of 1X PBS + 0.1% Tween 20.

5) In a white Optiplate (384 wells):



- 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, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).

Typical Product Data for Quality Control Assay



The data was generated using a 25 µL final volume in a white Optiplate-384 microplate and an EnVision-Alpha Reader 2103.

- Specificity:

All antibodies tested for cross reactivity with Anti-C-tag AlphaLISA Acceptor beads were referenced to C-tag at 10nM final probe concentration in the well (Tube E in the assay described above). Biotinylated probes were tested.

Protein	% Cross-reactivity
Biotin- GST	0
Biotin-His Tag peptide	0
Biotin-Flag peptide	0

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

http://www.perkinelmer.com/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

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