CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaLISA®

Anti- VHH Acceptor Beads

Product number:	AL180C	**Lot Number:**	3219817

Material provided: AlphaLISA Anti-VHH Acceptor Beads at 5 mg/mL in PBS, pH 7.4 supplemented with 0.05% Kathon as a preservative.

Product Format: AL180C: 250 µg, 50 µL, 500 assay points

AL180M: 5 mg, 1 mL, 10 000 assay points

AL180R: 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:	8/30/2023	**Document version:**	1

Product Information

Application: This product is intended for use in homogeneous Alpha assays for the capture of VHHs.

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 signal, minimum signal, S/B 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}:	0.32 nM
Min counts:	169 counts
Max counts:	32854 counts
S/B:	195

Titration Assay (Quality Control Procedure)

This protocol provides a means to verify product performance. The following reagents and materials are recommended.

Item	Suggested source
AlphaPlate™-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision®-Alpha Reader	Revvity Inc.
Anti-6xHis AlphaLisa Donor Beads	Revvity Inc.
VHH-cmyc-6His	NA
AlphaLISA PPI Buffer 5X	Revvity Inc.

Recommendations

- Alpha 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 PPI Buffer.
- 1X AlphaLISA PPI Buffer contains 50mM HEPES (pH 7.3),100mM NaCl, 0.5% Triton X-100, 0.5% BSA. 1X AlphaLISA PPI 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 a~~n~~ 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 PPI Buffer:
Add 1 mL of 5X AlphaLISA PPI Buffer to 4 mL Milli-Q® grade H_2O.

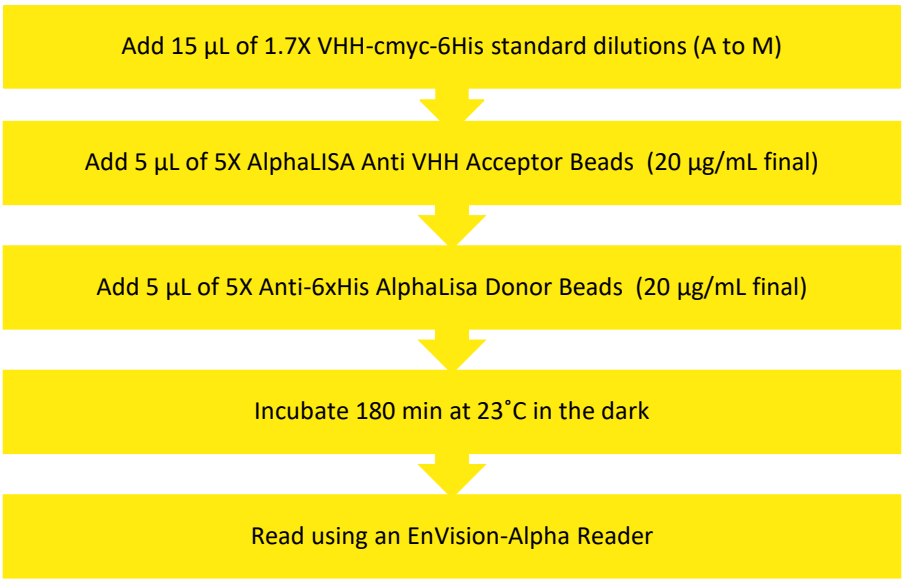
2) Preparation 1.7X VHH-cmyc-6His (20µl at 4µM) dilutions:
Dilute VHH-cmyc-6His to 400 nM stock solution
Prepare 1.7X dilutions in 1X AlphaLISA PPI Assay Buffer as follows:

Tube	*Volume of* VHH-cmyc-6His	Volume of 1X buffer (µL)	[VHH-cmyc-6His] (M) in 15 µL (1.7X)	[VHH-cmyc-6His] (M) in final assay volume (25 µL)
A	40 µL of predilution	279	5.1E-8	3.0E-8
B	60 µL of tube A	120	1.7E-8	1.0E-8
C	60 µL of tube B	140	5.1E-9	3.0E-9
D	60 µL of tube C	120	1.7E-9	1.0E-9
E	60 µL of tube D	140	5.1E-10	3.0E-10
F	60 µL of tube E	120	1.7E-10	1.0E-10
G	60 µL of tube F	140	5.1E-11	3.0E-11
H	60 µL of tube G	120	1.7E-11	1.0E-11
I	60 µL of tube H	140	5.1E-12	3.0E-12
J	60 µL of tube I	120	1.7E-12	1.0E-12
K	60 µL of tube J	140	5.1E-13	3.0E-13
L	60 µL of tube K	120	1.7E-13	1.0E-13
M	0	140	0	0

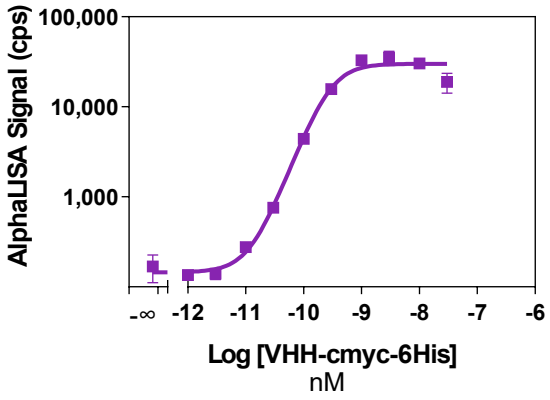
3) Preparation of 5X AlphaLISA Anti VHH Acceptor beads (100 µg/mL):
Add 10 µL of 5 mg/mL AlphaLISA Anti VHH Acceptor beads to 490µL of 1X AlphaLISA PPI Buffer.

4) Preparation of 5X Anti-6xHis AlphaLisa Donor Beads (100 µg/mL):
Keep the beads under subdued laboratory lighting. Add 10µL of 5 mg/mL Anti-6xHis AlphaLisa Donor Beads to 490 µL of 1X AlphaLISA PPI Buffer.

5) In a AlphaPlate-384 light gray microplate:



Typical Product Data



The signal was measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in the quality control procedure.

* 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 10 nM).

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

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