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CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaScreen®

Anti-6xHis Donor Beads

Product number:	AS116 D	**Lot Number:**	3395919

Material provided: Anti-6xHis Alpha Donor Beads at 5 mg/mL in PBS pH 7.4 supplemented with 0.05% Kathon as a preservative. The antibody used is a mouse monoclonal antibody.

Product Format: AS116D: 1 mg, 200µL, 2000 assay points

AS116M: 5 mg, 1000µL, 10 000 assay points

AS116R: 25 mg, 5000µL, 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:	April 16, 2025	**Document version:**	1

Product Information

Application: This product is intended for use in homogeneous Alpha assays to capture 6xHis-tagged targets.

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 EC_{50} 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}:	0.73 nM
Min counts:	569 counts
Max counts:	79071 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
Light Gray AlphaPlate-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
EnVision®-Alpha Reader	Revvity Inc.
AlphaLISA® Anti-hIgG Acceptor Beads	Revvity Inc.
Biotinylated-6His peptide	Revvity Inc.
AlphaLISA PPI 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 PPI Buffer.
- 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 PPI Buffer:
Add 2 mL of 5X AlphaLISA Universal Buffer to 8 mL Milli-Q® grade H_2O.

2) Preparation Biotinylated-6His dilutions:
Take one tube of Biotinylated-6His peptide (stock solution at 5 µM). Prepare dilution series in 1X AlphaLISA PPI Buffer as follows, changing the tip for each dilution:

Tube	Volume of Probe	Volume of buffer (µL)	Biotinylated-6His (M) in 15 µL	Biotinylated-6His (M) in (25 µL)
Predilution	5 µL of 5 µM	494	-	-
A	60 µL of predilution	120	1.67E-08	1.0E-08
B	60 µL of tube A	140	5.01E-09	3.0E-09

C	60 µL of tube B	120	1.67E-09	1.0E-09
D	60 µL of tube C	140	5.01E-10	3.0E-10
E	60 µL of tube D	120	1.67E-10	1.0E-10
F	60 µL of tube E	140	5.01E -11	3.0E-11
G	60 µL of tube F	120	1.67E-11	1.0E-11
H	60 µL of tube G	140	5.01E -12	3.0E-12
I	60 µL of tube H	120	1.67E-12	1.0E-12
J	60 µL of tube I	140	5.01E -13	3.0E-13
K	60 µL of tube J	120	1.67E-13	1.0E-13
L	60 µL of tube K	140	5.01E-14	3.0E-14
M	0	100	0	0
N	0	100	0	0
O	0	100	0	0
P	0	100	0	0

3) Preparation of 5X Streptavidin AlphaLISA Acceptor beads (100 µg/mL):
    a. Prepare just before use.
    b. Add 10 µL of 5 mg/mL Streptavidin AlphaLISA Acceptor beads to 490 µL of 1X AlphaLISA PPI Buffer. Mix briefly.

4) Preparation of 5X Anti-6xHis Alpha Donor beads (100 µg/mL):
    a. Prepare just before use and keep the beads **under subdued laboratory lighting**.
    b. Add 10 µL of 5 mg/mL Anti-6xHis Alpha Donor beads to 490 µL of 1X AlphaLISA PPI Buffer. Mix briefly.

5) In a light gray AlphaPlate-384:

Add 15 µL of probe dilution

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Add 5 µL of 5X Streptavidin AlphaLISA Acceptor Beads (20 µg/mL final)

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Add 5 µL of 5X Anti-6xHis Alpha Donor Beads (20 µg/mL final)

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Incubate 90 min at 23˚C in the dark

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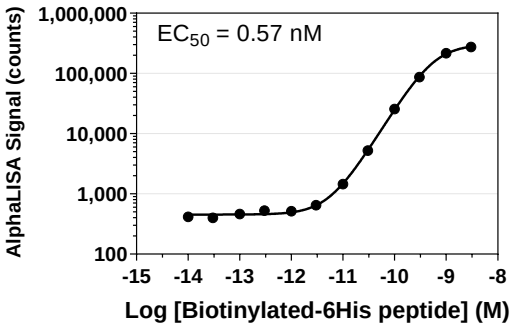
Read using EnVision-Alpha Reader

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.

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

AS116-R Rev 01

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