CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaLISA®

Anti- methyl-Histone H4 Arginine 3 (H4R3me) Acceptor Beads

Product number:	AL150C	**Lot Number:**	3211395

Material provided: Anti-methyl Histone H4 Arginine 3 (H4R3me) AlphaLISA Acceptor beads at 5 mg/mL in PBS pH 7.2, supplemented with 0.05% Kathon as a preservative. Source of the antibody: mouse monoclonal.

Product Format: AL150C: 250 µg, 50 µL, 500 assay points

AL150M: 5 mg, 1 mL, 10 000 assay points

AL150R: 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:	10/03/2023	**Document version:**	1

Product Information

Application: This product is designed to detect human histone H4 methylated at arginine 3 (H4R3me) in a homogeneous AlphaLISA® assay. Broad species cross-reactivity is expected based on sequence similarity.

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}:	14.27 nM
Min counts:	191 counts
Max counts:	257418 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.
Histone H4R3me2s peptide, biotinylated	AnaSpec, 65424
Histone H4 (1-21) peptide, biotinylated	AnaSpec, 62555
AlphaLISA® 5X Epigenetics Buffer 1 Kit	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.
- 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) Assay Buffer:
The Assay Buffer used for biotin-peptide dilution is 50 mM Tris-HCl pH 8.0.

2) Serial dilutions of biotin-peptide in Assay Buffer:
Prepare dilution series for each biotin-peptide as follows, changing tip for each dilution:

Tube	*Volume of* biotin-peptide	Volume of buffer (µL)	[Biotin-peptide] (M) in 10 µL
A	3 µL of 250 µM	247	3E-6
B	60 µL of tube A	120	1E-6
C	60 µL of tube B	140	3E-7
D	60 µL of tube C	120	1E-7

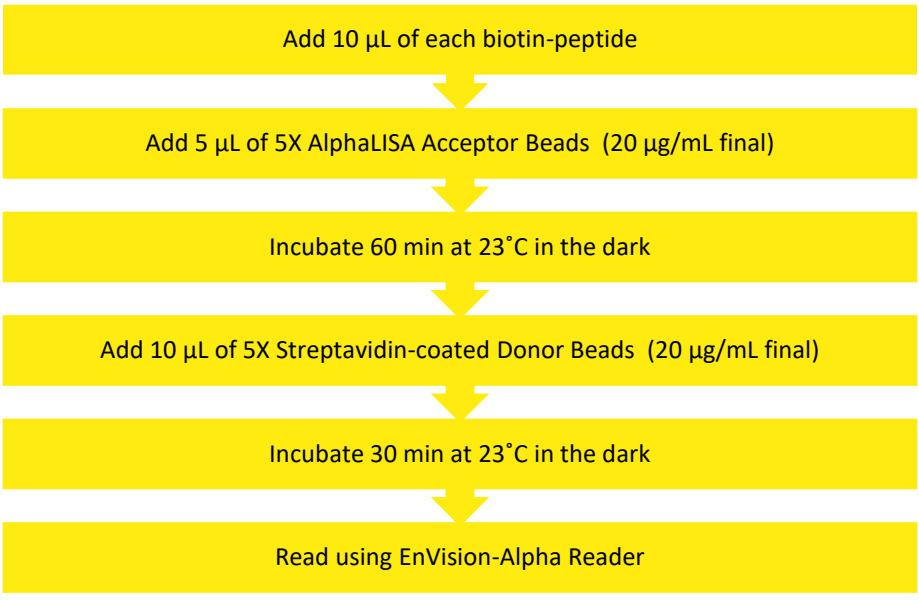
E	60 µL of tube D	140	3E-8
F	60 µL of tube E	120	1E-8
G	60 µL of tube F	140	3E-9
H	60 µL of tube G	120	1E-9
I	60 µL of tube H	140	3E-10
J	60 µL of tube I	120	1E-10
K	60 µL of tube J	140	3E-11
L	0	100	0

3) Preparation of 1X AlphaLISA Epigenetics Buffer 1:
Add 2.0 mL of AlphaLISA 5X Epigenetics Buffer 1 and 0.33 mL of AlphaLISA 30X Buffer Supplement to 7.67 mL H_2O. The cloudy appearance of the buffer is normal. Use the 1X Epigenetics Buffer within 16 hours.

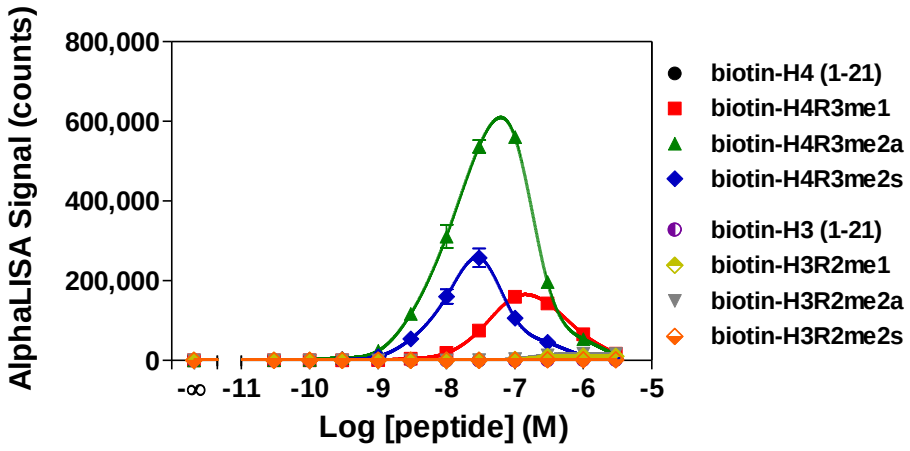
4) Preparation of 5X AlphaLISA Acceptor beads (100 µg/mL):
Add 10 µL of 5 mg/mL AlphaLISA Acceptor beads to 490 µL of 1X AlphaLISA Epigenetics Buffer 1

5) Preparation of 2.5X Streptavidin Donor Beads (50 µg/mL):
Keep the beads under subdued laboratory lighting. Add 10 µL of 5 mg/mL Streptavidin Donor beads to 990 µL of 1X AlphaLISA Epigenetics Buffer 1.

6) In a OptiPlate-384 microplate:



Typical **Product Data**



* Specificity of Anti-methyl-Histone H4 Arginine 3 (H4R3me) Acceptor Beads. Histone-derived peptides with different epigenetic marks were titrated. Signal was detected with an EnVision. The hook effect observed at higher peptide concentrations is typical of three-component assays and occurs when peptide concentrations exceed the binding capacity of the Alpha Donor and/or AlphaLISA Acceptor beads. Shifts in affinity might be observed in different enzymatic assay buffers. Signal generated by the following biotinylated peptides was not significantly different from that of the non-modified H3 (1-21) peptide: H3R8me1, H3R8me2a/s, H3R17me1, H3R17me2a/s, H3R26me1 and H3R26me2a/s (data not shown).

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

AL150-R Rev01

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