CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaLISA®

Anti-di-methyl-Histone H3 Lysine 36 (H3K36me2) Acceptor Beads for Full-length Histone H3 and Nucleosomes

Product number: AL152C **Lot Number:** 3365321

Material provided: Anti-di-methyl-Histone H3 Lysine 36 (H3K36me2) AlphaLISA Acceptor beads for Full-length Histone H3 and Nucleosomes at 5 mg/mL in PBS pH 7.2, supplemented with 0.05% Kathon as a preservative. Source of the antibody: rabbit monoclonal.

Product Format: AL152C: 250 µg, 50 µL, 500 assay points

AL152M: 5 mg, 1 mL, 10 000 assay points

AL152R: 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: September 26, 2024 **Document version:** 1

Product Information

Application: This Acceptor bead product is specifically designed to detect human histone H3 di-methylated at lysine 36 (H3K36me2) in a homogeneous AlphaLISA® assay using full length substrates such as recombinant histone H3 and native or recombinant nucleosomes. This product is not intended for enzymatic assays using short histone H3-derived peptides. For peptide assays, the AL123 anti-H3K36me2 Acceptor beads are recommended. 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}:	12.74 nM
Min counts:	289 counts
Max counts:	750114 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.
AlphaLISA® biotinylated anti-Histone H3 Antibody (C-ter)	Revvity Inc.
Recombinant Histone H3 (C110A)	Active Motif, 31207
Recombinant Histone H3K36me1 (MLA)	Active Motif, 31217
Recombinant Histone H3K36me2 (MLA)	Active Motif, 31218
Recombinant Histone H3K36me3 (MLA)	Active Motif, 31219
Poly-L-lysine	Sigma, P1399

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) Buffers:
Assay Buffer: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 0.01% Tween-20

High Salt Buffer: 50 mM Tris-HCl pH 7.4, 1 M NaCl, 0.1% Tween-20, 0.3% poly-L-lysine
Detection Buffer: 50 mM Tris-HCl pH 7.4, 300 mM NaCl, 0.1% Tween-20, 0.001% poly-L-lysine

2) Serial dilutions of full-length recombinant histone proteins (bare H3 and modified) in Assay Buffer:
Prepare dilution series for each recombinant histone protein as follows, changing tip for each dilution:

Tube	*Volume of* protein	Volume of buffer (µL)	[protein] (M) in 10µL
A	3 µL of 130 µM stock (bare H3) 4.8 µL of 65 µM stock (modified)	127 99.2	3E-6
B	30 µL of tube A	60	1E-6
C	30 µL of tube B	70	3E-7
D	30 µL of tube C	60	1E-7
E	30 µL of tube D	70	3E-8
F	30 µL of tube E	60	1E-8
G	30 µL of tube F	70	3E-9
H	30 µL of tube G	60	1E-9
I	30 µL of tube H	70	3E-10
J	30 µL of tube I	60	1E-10
K	30 µL of tube J	70	3E-11
L	0	60	0

3) Preparation of 5X AlphaLISA Acceptor Beads/biotinylated antibody mix (100 µg/mL and 5 nM, respectively):
Add 18 µL of 5 mg/mL AlphaLISA Acceptor beads and 9 µL of biotinylated antibody to 873 µL of Detection Buffer.

4) Preparation of 5X Streptavidin Donor Beads (100 µg/mL):
Keep the beads under subdued laboratory lighting. Add 18 µL of 5 mg/mL Streptavidin Donor beads to 882 µL of Detection Buffer.

5) In a OptiPlate-384 microplate:

Add 10 µL of full-length recombinant protein dilution

Add 5 µL of High Salt Buffer

Add 5 µL of 5X AlphaLISA Acceptor beads /Biotinylated Antibody Mix (20 µg/mL and 1 nM final, respectively)

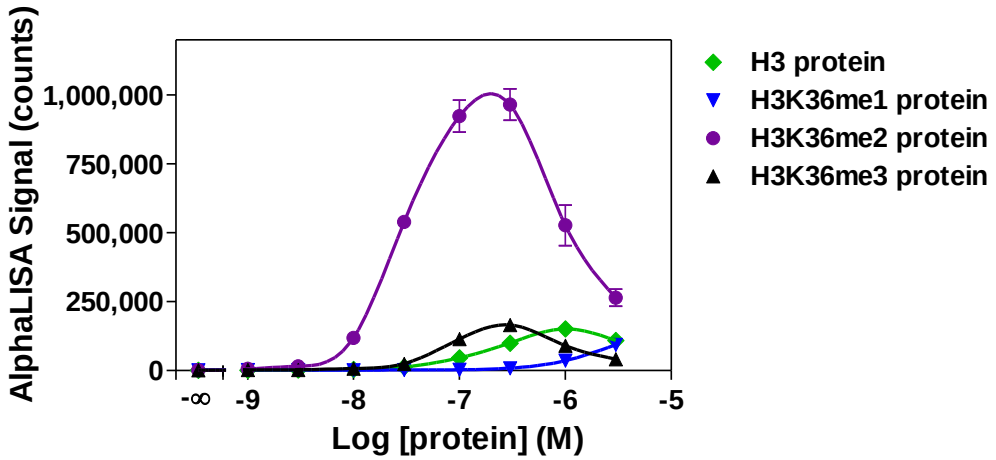
Incubate 60 min at 23˚C in the dark

Add 5 µL of 5X Streptavidin-coated Donor Beads (20 µg/mL final)

Incubate 30 min at 23˚C in the dark

Read using EnVision-Alpha Reader

Typical Product Data



* Specificity of the AL152 anti-di-methyl-Histone H3 Lysine 36 (H3K36me2) Acceptor Beads. Histone H3-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.

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

AL152-R Rev01

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