TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

Anti-methyl-Arginine Acceptor Beads

Product No.: **AL151C (250 µg)**
AL151M (5 mg)
AL151R (25 mg)

Lot No.: 2552000

Product Formats

Catalog #	Size	Volume	Assay points*
AL151C	250 µg	0.05 mL	500
AL151M	5 mg	1 mL	10 000
AL151R	25 mg	5 mL	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.

Manufacturing Date: March 6, 2019

Product Information

Description: Anti-methyl-Arginine AlphaLISA Acceptor beads at 5 mg/mL in PBS pH 7.2, supplemented with 0.05% Proclin-300 as a preservative. Source of the antibody: mouse monoclonal.

Application: This product is designed to detect histones methylated at arginine residues in a homogeneous AlphaLISA® assay. Broad cross-reactivity is expected based on sequence similarity.

Storage: Store in the dark at 4˚C.

Stability: This product is stable for at least 12 months from the manufacturing date when stored in its original packaging at the recommended storage conditions.

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Quality Control

Lot-to-lot consistency is confirmed by a Quality Control AlphaLISA titration assay read on an EnVision® Alpha HTS instrument. Maximum signal and EC_{50} value are determined using the biotin-H4R3me2s peptide. Minimum signal is derived from the non-modified biotin-H4 (1-21) peptide at the concentration giving the specified maximum signal. We certify that this product meets our quality release criteria. Maximum counts may vary between lots and depending on assay conditions.

Maximum signal: 615,745 counts
Minimum signal: 161 counts
EC_{50}: 51.56 nM

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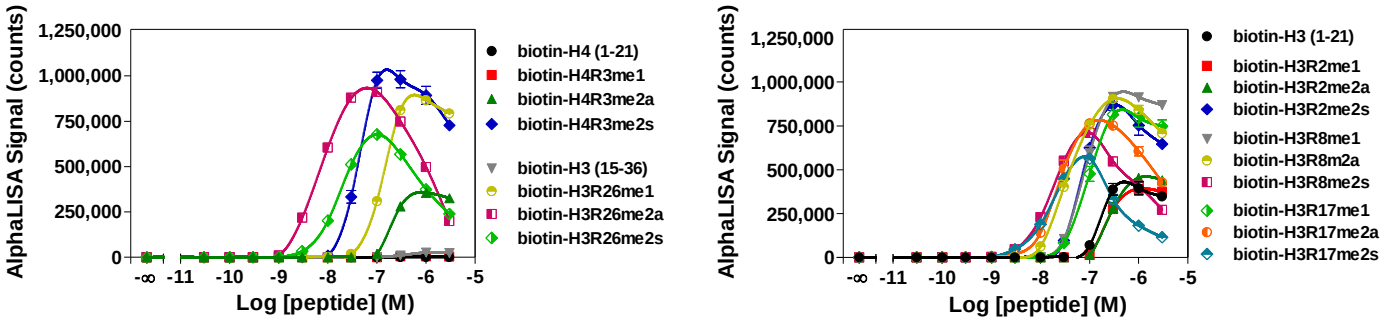
Typical Data



Specificity of Anti-methyl-Arginine Acceptor Beads. Histone derived peptides with different epigenetic marks were titrated in 50 mM Tris-HCl pH 8.0 assay buffer. *Left Panel:* Biotinylated Histone H4 (1-21) and Histone H3 (15-36) peptides. Right panel: Biotinylated Histone H3 (1-21) peptides. Signal was detected with an EnVision Alpha HTS instrument 2102. 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 Streptavidin Donor and/or AlphaLISA Acceptor beads. Sensitivity may vary in other assay buffers.

A technical note presenting the optimization of an AlphaLISA PRMT4 (CARM1) histone H3 arginine methyltransferase assay using anti-methyl arginine Acceptor beads is available on our website at www.perkinelmer.com/epigenetics. The anti-methyl-arginine AlphaLISA Acceptor beads can also be used for measuring the activity of PRMT5 using a biotinylated Histone H4 (1-21) peptide substrate.

Peptide Titration Assay

Peptide titration provides a means to verify product performance. It is used as our Quality Control release test. The following reagents and materials are recommended:

Item	Supplier	Catalog #
AlphaScreen® Streptavidin Donor Beads	PerkinElmer	6760002S (1 mg) 6760002 (5 mg) 6760002B (50 mg)
Histone H4R3me2s peptide, biotinylated	AnaSpec	65424
Histone H4 (1-21) peptide, biotinylated	AnaSpec	62555
AlphaLISA® 5X Epigenetics Buffer 1 Kit*	PerkinElmer	AL008C (10 mL Kit) AL008F (100 mL Kit)
White opaque OptiPlate™-384	PerkinElmer	6007290
TopSeal™-A Adhesive Sealing Film	PerkinElmer	6050195
EnSpire® or EnVision® Multilabel Plate Reader with Alpha option	PerkinElmer	-

*The two-component AlphaLISA 5X Epigenetics Buffer 1 Kit includes AlphaLISA 5X Epigenetics Buffer 1 and AlphaLISA 30X Buffer Supplement. The 1X AlphaLISA Epigenetics Buffer 1 may appear cloudy. However, this will not affect assay performance. The 1X Epigenetics Buffer 1 should be used within 16 hours.

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These microplates can also be used with this product:

Item	Recommended Assay Volume	Supplier	Catalog #
White opaque OptiPlate-96	100 µL	PerkinElmer	6005290
White ½ AreaPlate-96	50 µL	PerkinElmer	6005560
Light gray AlphaPlate™-384	25 µL	PerkinElmer	6005350
ProxiPlate™-384 Plus	12.5 µL	PerkinElmer	6008280
Light gray AlphaPlate-1536	5 - 10 µL	PerkinElmer	6004350

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 prewet the tip.
- AlphaScreen Donor beads are light-sensitive. All steps using Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures. All other assay reagents can be used under normal light conditions.
- Sodium azide should not be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.
- Centrifuge the 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 H_2O (18 MΩ•cm) to prepare the 1X AlphaLISA Epigenetics Buffer 1.
- When diluting peptides, change tips after each dilution. When loading reagents in the assay microplate, change tips after each reagent addition and between each set of reagents.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with a 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 EnSpire® or EnVision Multilabel Plate Reader equipped with the ALPHA option using the AlphaScreen standard settings (i.e. Total Measurement Time: 550 ms, Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).

Quality Control Protocol

PLEASE READ RECOMMENDATIONS ON PAGE 3 BEFORE USE

This titration protocol is designed for 12 dilutions of 2 peptides (biotin-H4R3me2s and non-modified biotin-H4 (1-21) peptides) with triplicate determinations. Peptide concentrations are indicated for a 10 µL volume. Final concentration of Alpha Donor and AlphaLISA Acceptor beads in the 25 µL total assay volume is 20 µg/mL.

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:

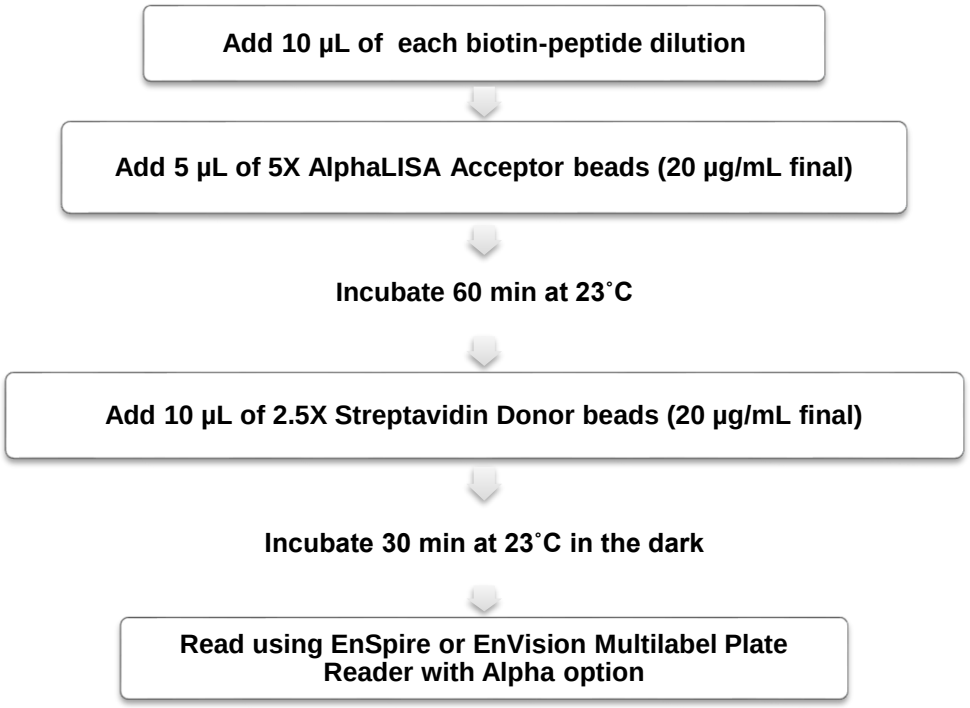
Tube	Volume of biotin-peptide	Volume of Assay Buffer (µL)	[biotin-peptide] (M) in 10 µL
A	3 µL of 250 µM stock	247	3.0E-6
B	60 µL of tube A	120	1.0E-6
C	60 µL of tube B	140	3.0E-7
D	60 µL of tube C	120	1.0E-7
E	60 µL of tube D	140	3.0E-8
F	60 µL of tube E	120	1.0E-8
G	60 µL of tube F	140	3.0E-9
H	60 µL of tube G	120	1.0E-9
I	60 µL of tube H	140	3.0E-10
J	60 µL of tube I	120	1.0E-10
K	60 µL of tube J	140	3.0E-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 1 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 white opaque OptiPlate-384 microplate:



Please visit our website for additional information on the AlphaLISA technology at www.perkinelmer.com/AlphaTech.

This product is not for resale or distribution except by authorized distributors.

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