TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

Biotinylated Anti-Histone H3 (C-ter) Antibody

Product No.: **AL118C (2 µg)**
AL118M (40 µg)
AL118R (200 µg)

Lot No.: 2667285

Product Formats

Catalog #	Size	Volume	Assay points*
AL118C	2 µg	25 µL	500
AL118M	40 µg	500 µL	10 000
AL118R	200 µg	2500 µL	50 000

* The number of assay points is based on an assay volume of 25 µL using a final antibody concentration of 1 nM in 384-well format.

Manufacturing Date: December 17, 2019

Product Information

Description: Biotinylated rabbit monoclonal antibody recognizing the carboxy-terminal (C-ter) sequence of human histone H3. Broad species cross-reactivity is expected based on sequence similarity.

Application: This product is designed to be used with AlphaLISA® Epigenetics Acceptor beads to detect modified full-length histone H3 in homogeneous AlphaLISA assays.

Storage Buffer: PBS pH 7.4, 0.1% Tween-20, and 0.05% sodium azide as a preservative.

Molecular Weight: 160 000

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.

Storage Conditions: Store at 4°C.

Safety Note: The storage buffer contains sodium azide. Disposal of all waste should be in accordance with local regulations.

Quality Control

The QC release specifications are based on spectrophotometric analysis of the labeled antibody. We certify that these results meet our quality release criteria.

Labeling Ratio: 7.42 biotin/Ab

Concentration: 500 nM (80 µg/mL)

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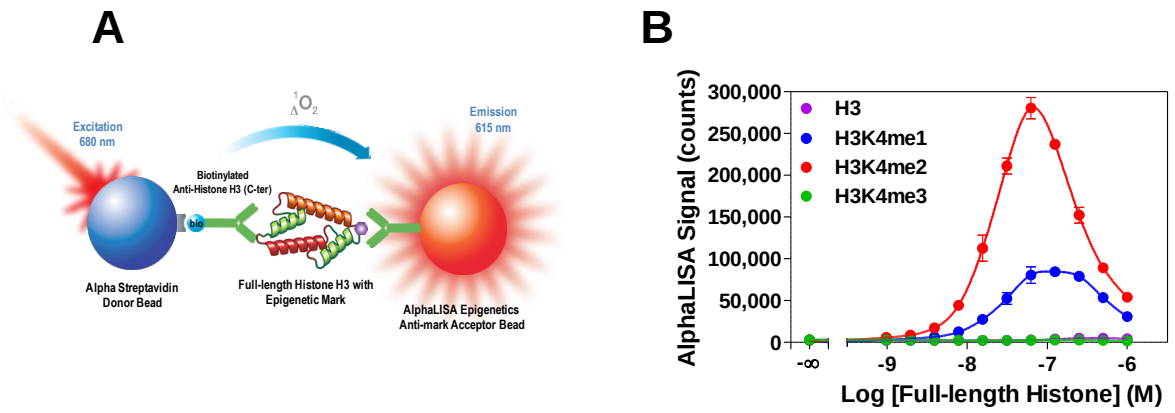
Typical Data



Full-length Histone H3 Titration Assay. A) Schematic representation of the AlphaLISA detection of a modified full-length histone H3 using the biotinylated anti-histone H3 (C-ter) antibody and anti-epigenetic mark AlphaLISA Acceptor beads. B) Serial dilutions of recombinant full-length histone H3 bearing different epigenetic marks were detected using the biotinylated anti-histone H3 antibody and anti-H3K4me1-2 Acceptor beads. Signal was detected with an EnVision 2103. The hook effect observed at higher protein concentrations is typical of a multi-component assay and occurs when protein concentrations exceed the binding capacity of the biotinylated anti-histone H3 (C-ter) antibody and/or AlphaLISA Acceptor beads.

A Technical Note presenting the optimization of an AlphaLISA SET7/9 histone H3 lysine methyltransferase assay using the biotinylated anti-histone H3 (C-ter) antibody is available on our website at www.perkinelmer.com/epigenetics.

Full-length Histone H3 Titration Assay

Protein titration provides a means to verify product performance. The following reagents and materials are recommended:

Item	Supplier	Catalog #
AlphaScreen® Streptavidin Donor Beads	PerkinElmer	6760002S (1 mg) 6760002 (5 mg) 6760002B (50 mg)
Anti-methyl-Histone H3 Lysine 4 (H3K4me1-2) Acceptor Beads*	PerkinElmer	AL116C (250 µg) AL116M (5 mg) AL116R (25 mg)
Recombinant Histone H3 (C110A)	Active Motif	31207
Recombinant Histone H3 Dimethyl Lys4 (H3K4me2)*	Active Motif	31209
White opaque OptiPlate™-384	PerkinElmer	6007290
TopSeal™-A Adhesive Sealing Film	PerkinElmer	6050195
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer	-

* The biotinylated anti-histone H3 (C-ter) antibody can also be used in conjunction with other AlphaLISA Epigenetics Acceptor beads. The full-length control histone required for the titration assay should be substituted accordingly.

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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

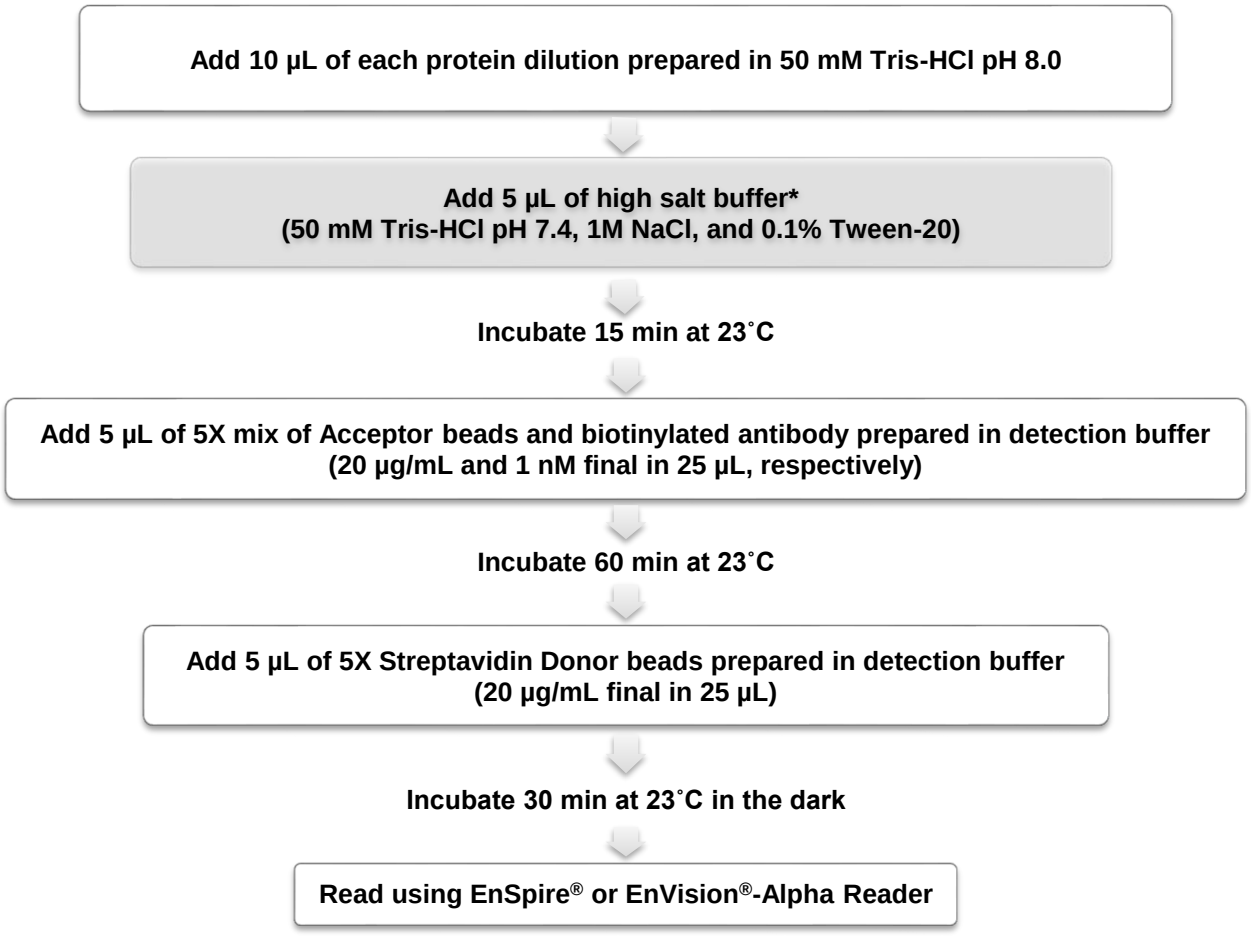
- AlphaScreen Donor beads are light-sensitive. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Centrifuge tubes briefly to improve recovery of content. Resuspend Alpha beads by vortexing gently before use.
- 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.
- Use Milli-Q® grade H_2O (18 MΩ•cm).
- When diluting full-length histone H3 proteins, 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.
- The AlphaLISA signal is detected with an EnSpire or EnVision Multilabel 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%).
- Total signal varies with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for all plates.

Protocol

PLEASE READ RECOMMENDATIONS BEFORE USE

This protein titration assay includes 12 serial dilutions with triplicate determinations. Protein concentrations are indicated for a 10 µL volume. Beads and biotinylated antibody are diluted in detection buffer containing 50 mM Tris-HCl pH 7.4, 300 mM NaCl, 0.1% Tween-20, and 0.001% poly-L-lysine. Final concentrations, in the 25 µL assay volume, of both Alpha beads and biotinylated antibody are 20 µg/mL and 1 nM, respectively.

In a white opaque OptiPlate-384 microplate:



* The formulation of the high salt buffer might require optimization for the detection of other epigenetic marks.

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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