

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.:** 2524696

### 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 18, 2018

### 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.

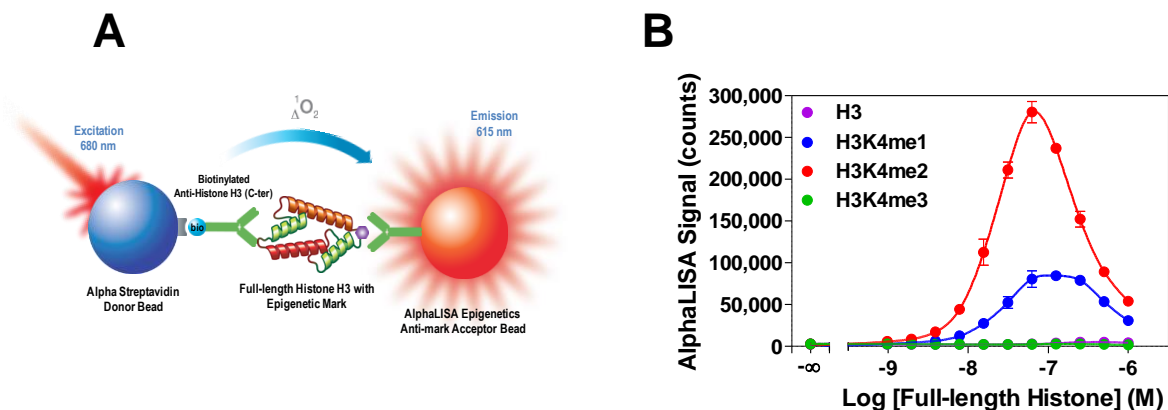
### Quality Control

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:** 5.89 biotin/Ab

**Concentration:** 500 nM (80 µg/mL)

## 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](http://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.

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

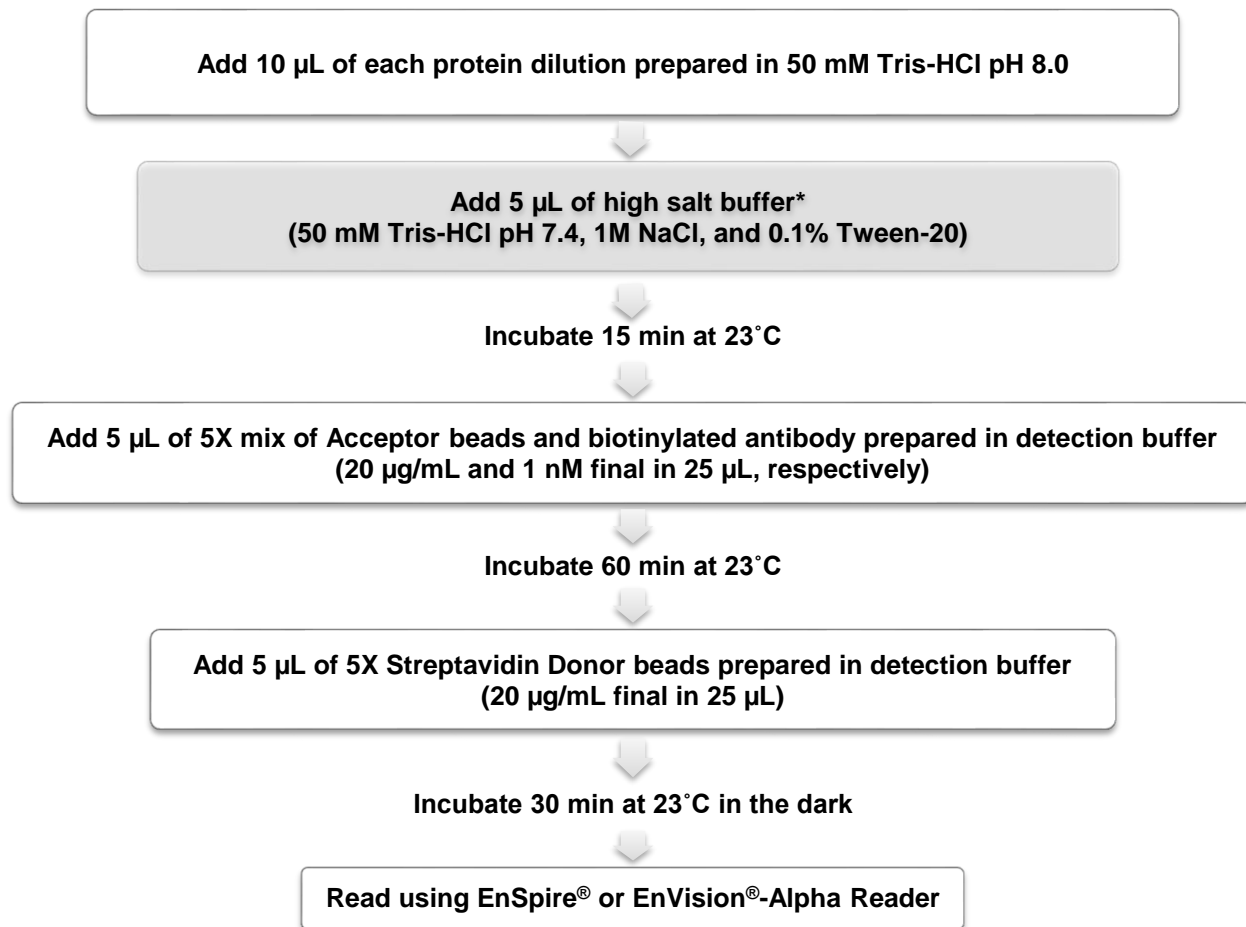
- AlphaScreen Donor beads are light-sensitive. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Centrifuge tubes briefly to improve 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<sub>2</sub>O (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](http://www.perkinelmer.com/AlphaTech).

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

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