

AlphaLISA®

Biotinylated Anti-Histone H3 (C-ter) Antibody**Product number:** AL118C **Lot Number:** 3323535**Material provided:** Anti-acetyl-p53 Lysine 382 (p53K382ac) 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:**
AL118C: 2 µg, 25 µL, 500 assay points
AL118M: 40 µg, 500 µL, 10 000 assay points
AL118R: 200 µg, 2500 µL, 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: 08/01/24 **Document version:** 1**Product Information****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.**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

Labeling Ratio: 7.67 biotin/Ab
Concentration: 500 nM (80 µg/mL)

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.
Anti-methyl-Histone H3 Lysine 4 (H3K4me1-2) Acceptor Beads*	Revvity Inc.
Recombinant Histone H3 (C110A)	Active Motif, 31207
Recombinant Histone H3 Dimethyl Lys4 (H3K4me2)*	Active Motif, 31209

* 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.

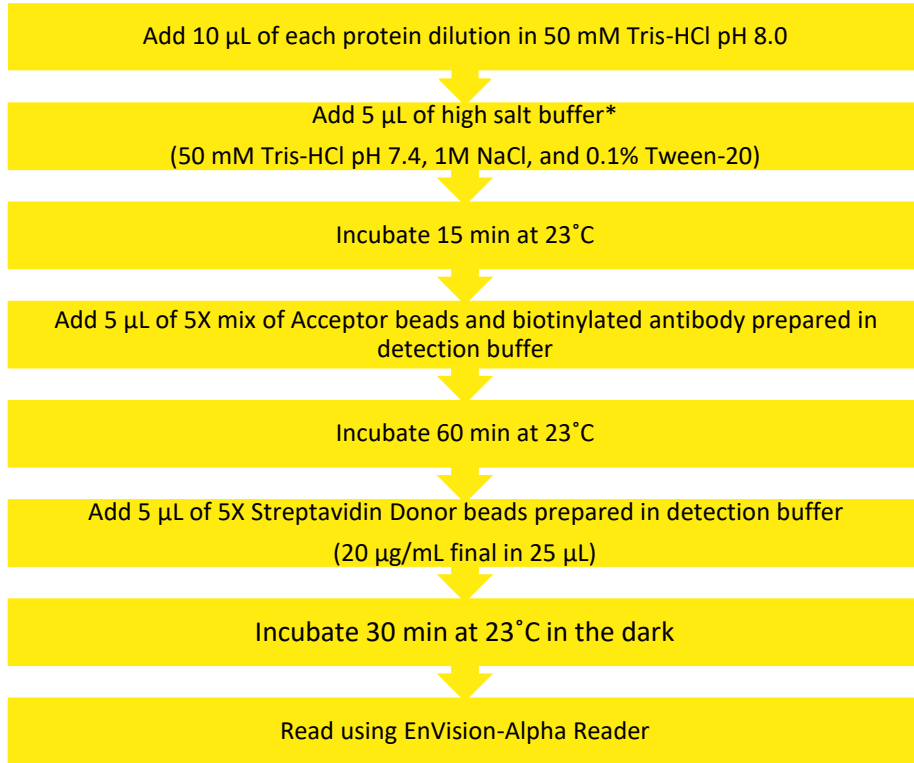
Recommendations

- 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

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 OptiPlate-384 microplate:



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

formulation of the high salt

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

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