TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

TruHits Kit

Product No.: **AL900D**
AL900M

Lot No.: 2652539

Product Formats

Catalog #	Assay points*
AL900D	1 000
AL900M	10 000

* The number of assay points is based on an assay volume of 25 µL using a final bead concentration of 10 µg/mL in 384-well format.

Manufacturing Date: November 14, 2019

Kit components:

Component	AL900D	AL900M
AlphaLISA BSA-biotin Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	1 x 50 µL at 5 mg/mL (AL900AD)	1 x 500 µL at 5 mg/mL (AL900AM)
Streptavidin Alpha Donor beads stored in 25 mM Hepes, 100 mM NaCl, 0.05% Kathon, pH 7.4	1 x 50 µL at 5 mg/mL (6760002D)	1 x 500 µL at 5 mg/mL (6760002M)

Product Information

Description: The AlphaLISA Acceptor beads are coated with biotinylated bovine serum albumin (BSA). The Alpha Donor beads are coated with streptavidin.

Application: This product is designed as a tool for AlphaLISA users to identify false positives in AlphaLISA screening assays.

Storage: Store in the dark at 4ºC.

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

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Quality Control

Lot-to-lot consistency is confirmed by a Quality Control one point assay read on an EnVision® HTS Alpha instrument (see protocol below). We certify that the results meet our quality release criteria. *Note: maximum counts will vary depending on assay conditions as well as between lots. This variation has no impact on assay quality.*

Maximum signal: 640,129 counts
Background: 544 counts

Description of the AlphaLISA TruHits Assay

The AlphaLISA TruHits kit is designed as a tool for AlphaLISA users to identify false positives in AlphaLISA HTS assays early in the screening process. Some compounds can interfere with the detection part of the assay, thereby artificially reducing AlphaLISA signal.

This kit includes AlphaLISA BSA-biotin Acceptor beads and Streptavidin Alpha Donor beads which interact together to generate an AlphaLISA signal. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm. The AlphaLISA TruHits kit allows the identification of inner filters, light scatterers (insoluble compounds), singlet oxygen quenchers and biotin mimetics interfering with the AlphaLISA signal, and thus facilitates the detection of false positives. However, TruHits kits do not allow systematic identification of all interfering compounds. Pan-assay interfering substances (PAINS) such as rhodanines will not cause a decrease in TruHits AlphaLISA signal.

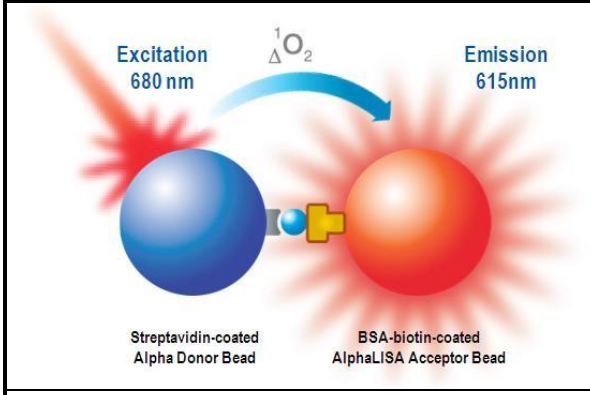
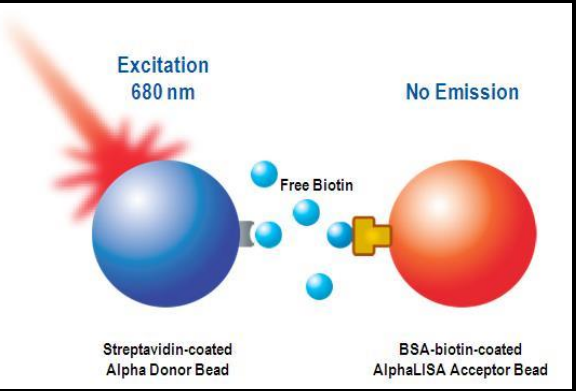
One Point Assay (Quality Control Protocol)

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

Item	Suggested Source	Catalog #
Biotin	Sigma	B4501
(1X) Phosphate-Buffered Saline (PBS), pH 7.2	Invitrogen	20012
White OptiPlate™-384	PerkinElmer	6007290
TopSeal™-A Adhesive Sealing Film	PerkinElmer	6050195
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer	-

Quality Control Protocol

This protocol provides a method to verify kit performance and is not representative of an assay. The maximum and background signals represent an average of 6 replicates. Background signal is obtained in the presence of free biotin. Final concentration of AlphaLISA BSA-biotin Acceptor beads and Streptavidin Alpha Donor beads is 10 µg/mL, in the 25 µL final assay volume.

Without Biotin	With Biotin
	
Maximum signal	Background

1) Preparation of 1.7X Biotin (17 µM):
Add 17 µL of 500 µM Biotin stock solution to 483 µL of 1X PBS, pH 7.2.

2) Preparation of 5X Streptavidin Alpha Donor beads (50 µg/mL):
Keep the beads under subdued laboratory lighting.
Add 5 µL of 5 mg/mL Alpha Donor beads to 495 µL of 1X PBS, pH 7.2.

3) Preparation of 5X AlphaLISA BSA-biotin Acceptor beads (50 µg/mL):
Add 5 µL of 5 mg/mL AlphaLISA Acceptor beads to 495 µL of 1X PBS, pH 7.2.

4) In a microtube:

Add 300 µL of 1X PBS, pH 7.2

Add 300 µL of 1.7X Biotin (10 µM final)

Add 100 µL of 5X Alpha Donor beads (10 µg/mL final)

Add 100 µL of 5X AlphaLISA Acceptor beads (10 µg/mL final)

Incubate 30 min at 23ºC in the dark

5) In a white opaque OptiPlate-384 microplate:

Distribute 25 µL in 6 replicates (-/+ biotin)

Incubate 10 min at 23ºC in the dark

Read using EnSpire or EnVision Multilabel Alpha Reader

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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.
- Alpha 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.
- Sodium azide should not be added to 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.
- When reagents are added in the microplate, make sure the liquids are at the bottom of the well.
- 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 Alpha-enabled EnSpire or EnVision Multilabel Reader using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm, Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).

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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