TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

AlphaLISA TruHits Biotin-Free Kit

Product number: AL901 D/M

Lot number: sample

Manufacturing date:

Caution: For Laboratory Use. A research product for research purposes only.

Product Information

Application: The 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 artificially reduce AlphaLISA signal by interfering with the technology itself. AlphaLISA Biotin-free TruHits kit contains Digoxigenin Acceptor beads and Anti-Digoxigenin Fab Fragment Donor beads at 5 mg/mL in PBS pH 7.2 supplemented with 0.05% Proclin-150 as a preservative.

Formats:

Catalog #	Assay Points*
AL901D	1 000
AL901M	10 000

*The number of assay points is based on an assay volume of 25 µL in384-well assay plates using a final bead concentration of 20 µg/mL.

Sensitivity: Average Maximal signal: 940 000 counts at 20µg/mL beads*

*As determined on an EnVision® Multilabel Plate Reader with Alpha option 2103.

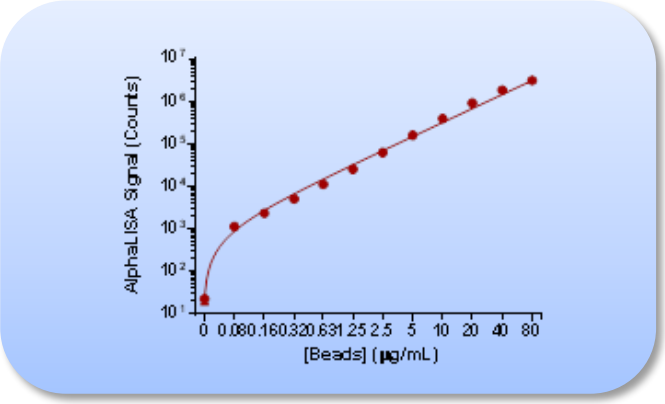

Figure. 1. Typical assay curve. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader with Alpha option 2103. The curve was obtained by mixing acceptor and donor beads at increasing concentrations. To insure linearity, maximum signals were considered at 20µg/mL beads.

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Storage: Store kit in the dark at +4˚C.

Stability: This product is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum signals were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. 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. Maximum signal is at 20 μg/mL beads while minimum signal is at 0.08 μg/mL.

Maximal signal: 503975 counts

Minimum signal: 515 counts

Description of the AlphaLISA Biotin-Free 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, when working with biotin-rich media. This kit includes AlphaLISA Digoxigenin TruHits Acceptor and Anti-Digoxigenin Fab Fragment 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 Biotin-Free TruHits kit allows the identification of light scattering molecules (insoluble compounds), singlet oxygen quenchers and Digoxigenin 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.

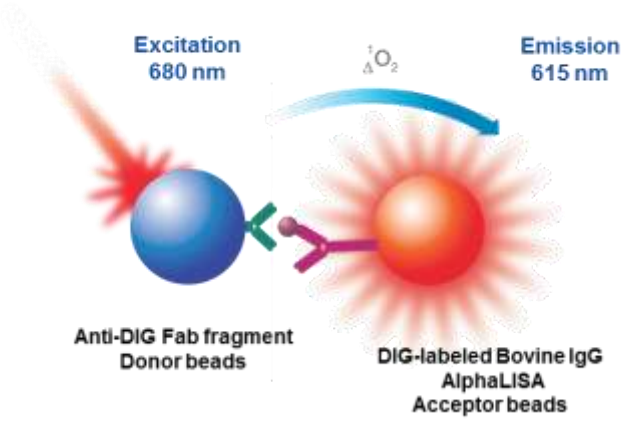

Figure 2. AlphaLISA Biotin-Free TruHit Assay principle.

Precautions

- Anti-Digoxigenin Fab Fragment Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. 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.
- The DIG labeled anti-analyte antibody is toxic. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL901D (1 000 assay points***)	AL901M (10 000 assay points***)
AlphaLISA Digoxigenin TruHits Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	100 µL @ 5 mg/mL (1 brown tube, white cap)	1000 µL @ 5 mg/mL (1 brown tube, white cap)
Anti-Digoxigenin Fab Fragment Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4	100 µL @ 5 mg/mL (1 brown tube, black cap)	1000 µL @ 5 mg/mL (1 brown tube, black cap)

Recommendations

- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- 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.
- 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 pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute the buffer.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to 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 Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment. The standard curve should be performed in the Immunoassay buffer for serum and/or plasma samples.

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer Inc.	6050185
EnVision®-Alpha Reader	PerkinElmer Inc.	-

Quality Control Protocol

This protocol provides a method to verify kit performance and is not representative of an assay.

The protocol described below is for one standard curve (36 wells)

1) Preparation of assay buffer

Add 2mL of 5X Universal buffer to 8mL of water.

2) Preparation of beads standard dilutions:

Prepare bead dilutions as follows (change tip between each standard dilution) using AlphaLISA Digoxigenin Acceptor Beads (5 mg/mL) and Alpha Anti-Dig Donor Beads (5 mg/mL) in 1X Universal buffer. (Change tip between each standard dilution):

Tube	Vol. of beads (µL)	Vol. of diluent (µL) *	[beads] in standard curve (ug/mL)
A	3.2µL of TruHits acceptor and 3.2µL of Donor beads*	194	80
B	100 µL of tube A	100	40
C	100 µL of tube B	100	20
D	100 µL of tube C	100	10
E	100 µL of tube D	100	5
F	100 µL of tube E	100	2.5
G	100 µL of tube F	100	1.25
H	100 µL of tube G	100	0.63
I	100 µL of tube H	100	0.32
J	100 µL of tube I	100	0.16
K	100 µL of tube J	100	0.08
L	0	100	0

* Donor beads are sensitive to light. Dilutions are performed under subdued or green light.

3) In a white Optiplate (384 wells): Add 25µL of each dilution of beads into 3 wells.

4) Cover plate with TopSeal.

5) Incubate plate for 1 hour at 23^{o}C in an incubator.

6) Read plate using Envision Multilabel Reader with Alpha Option 2103

   a. AlphaLISA signal is detected using an EnVision Multilabel Reader equipped with the Alpha option using the following settings: Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: 640as (Barcode# 444), Emission Filter: Wavelength 570nm, bandwidth: 100nm, Transmittance 75%, (Barcode# 244).

Suggested Materials and Instrumentation

Please visit our website www.perkinelmer.com/AlphaTech

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

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