TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

αSMA AlphaLISA Detection Kit

Product No.: AL3143

Contents

Product Information

Application: This kit is designed for the quantitative determination of αSMA (Smooth Muscle Actin) using a homogeneous no wash AlphaLISA assay.

Kit contents: The kit contains 6 components: AlphaLISA Acceptor beads coated with anti-αSMA Antibody, Streptavidin-coated Donor beads, Biotinylated anti-αSMA antiboody, Lyophilized αSMA positive control, 5X AlphaLISA Lysis buffer and 10X AlphaLISA Immunoassay Buffer.

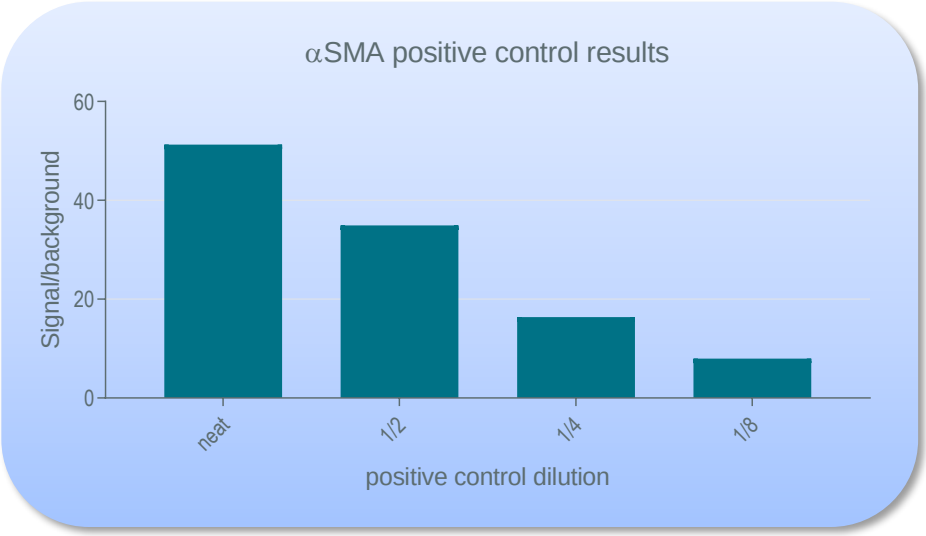


Figure 1. Typical positive control lysate diluted in lysis buffer. The data was generated using a white Alphaplate™-384 microplate and read on an EnVision™ Multilabel Plate Reader. Total signal, signal/background window, and sensitivity may vary with other instruments.

Storage: Store kit in the dark at 4 ºC.
For reconstituted analyte, aliquot and store at -20 ºC. Avoid freeze-thaw cycles.

Stability: This kit is stable for at least 12 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

After tissue injury, TGF-beta locally released by inflammatory cells activates resident fibroblasts or quiescent HSCs (hepatic stellate cells). This leads to their differentiation into myofibroblasts, whose role is to migrate into the damaged tissue and synthesize ECM (extracellular matrix) components to repair the wound. Myofibroblasts are characterized by de novo expression of alpha-SMA, which is incorporated into actin stress fibers and confers a high contractile activity to the cells. Chronic tissue injury leads to persistent de novo formation of myofibroblasts (alpha-SMA+), excessive contraction, and deposition of ECM, eventually leading to tissue fibrosis.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in cell lysate in a highly sensitive, reproducible and user-friendly mode. In this AlphaLISA assay, a biotinylated anti-αSMA antibody binds to the streptavidin coated AlphaLISA Donor beads, while the anti-αSMA antibody is conjugated to AlphaLISA Acceptor beads. In the presence of αSMA, the beads come into proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer within the Acceptor beads, resulting in emission with λ_{max} at 615 nm (Figure 2).

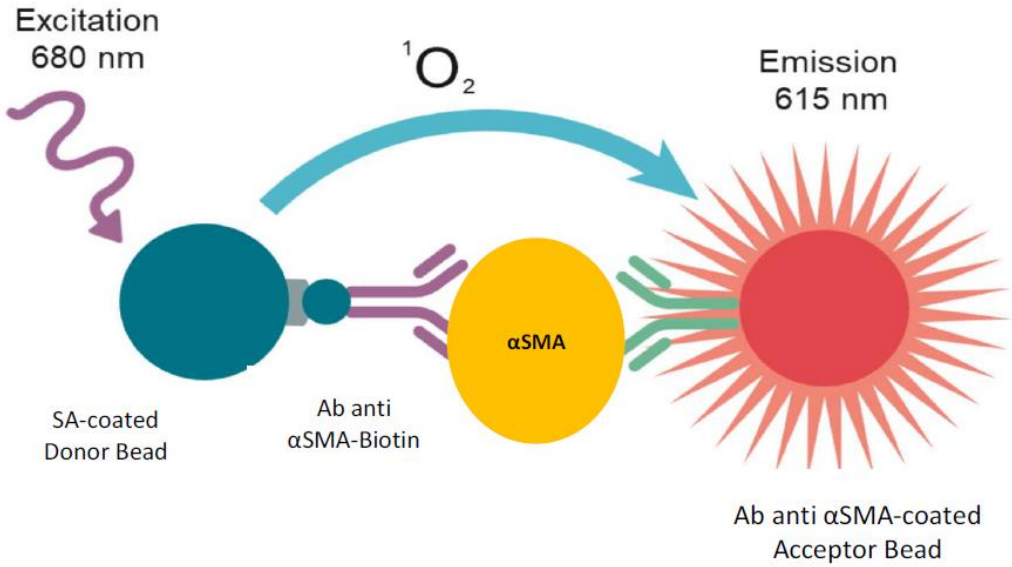


Figure 2. AlphaLISA -αSMA Detection Assay Principle.

Precautions

- The Alpha 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.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The biotinylated anti-αSMA antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

Kit components	AL3143HV 100 assay points****	AL3143C 500 assay points****	AL3143F 5000 assay points****
AlphaLISA Anti-αSMA Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	50 µL @ 5 mg/mL (1 brown tube, white cap)	500 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon CG/ICP, pH 7.4	80 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	2 mL @ 5 mg/mL (1 brown tube, black cap)
Biotinylated Anti-αSMA Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	60 µL @ 500 nM (1 tube, black cap)	150 µL @ 500 nM (1 tube, black cap)	1.5 mL @ 500 nM (1 tube, black cap)
Lyophilized αSMA positive control*	1 tube, clear cap	1 tube, clear cap	1 tube, clear cap
AlphaLISA Lysis Buffer (5X)**	10 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle
AlphaLISA Immunoassay Buffer (10X)***	10 mL, 1 small bottle	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute lyophilized analyte in 300 µL Milli-Q® grade H_2O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped 0.5 mL polypropylene vials and stored at -20 °C for future experiments. The aliquoted analyte at -20 °C is stable up to 30 days. Avoid freeze-thaw cycles. Additional vials can be ordered separately (cat # AL3143S).

** Extra buffer can be ordered separately (cat # AL003C: 10 mL, cat # AL003F: 100 mL

*** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

**** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 384-well assay plates using the kit components at the recommended concentrations.

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. Note that sodium azide from the biotinylated anti-αSMA antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

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

Recommendations

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- 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 (2000*g*, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O to dilute 10X AlphaLISA Immunoassay Buffer and 5X AlphaLISA Lysis Buffer and to reconstitute the lyophilized analyte.
- When diluting the samples, change tips between each 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 in place.
- The AlphaLISA signal is detected with an EnVision Multilabel Plate Reader equipped with the Alpha option 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%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.

Assay Procedure

- The protocol described below is an example for generating assay in a 50 µL final assay volume (triplicate determinations), the positive control is provided in order to validate the detection efficacy. The protocols include testing samples in 500 wells. If different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.

		Volume				
Format	**# of data points**	**Final**	**Sample**	**MIX AlphaLISA AccBeads + biotinylated Ab**	**SA-Donor beads**	**Plate recommendation**
AL3143HV	100	100 µL	10 µL	10 µL	50 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL3143C	250	100 µL	10 µL	40 µL	50 µL	White OptiPlate-96 (cat # 6005290)
	500	50 µL	5 µL	20 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL3143F	5 000	50 µL	5 µL	20 µL	25 µL	½ Area AlphaPlate-96 (cat # 6002350) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	8 µL	10 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	4 µL	5 µL	Light gray AlphaPlate-1536 (cat # 6004350)

Reagent preparation:

1) Preparation of 1X AlphaLISA Lysis Buffer:
   Add 5 mL of 5X AlphaLISA Lysis Buffer to 20 mL Milli-Q® grade H_2O.

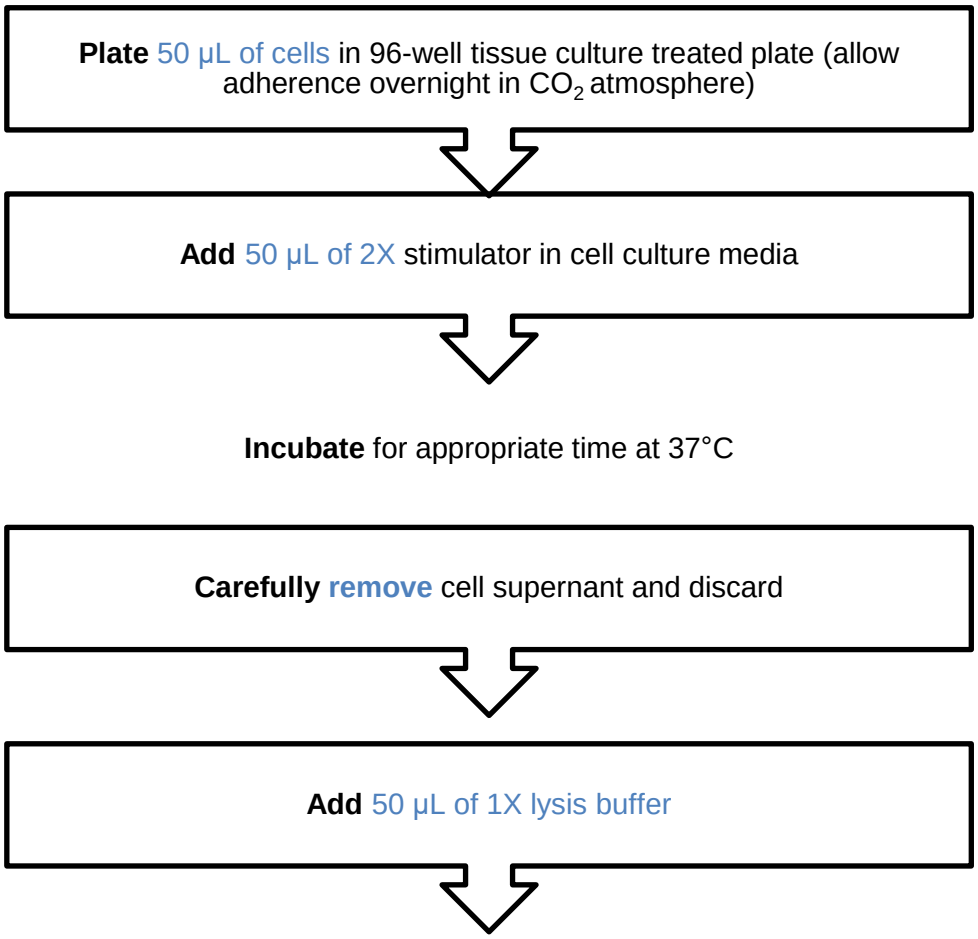
2) Preparation of 1X AlphaLISA Immunoassay Buffer:
   Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL Milli-Q® grade H_2O.

3) Preparation of αSMA positive control:
   a. Reconstitute lyophilized -αSMA in 300 µL Milli-Q® grade H_2O. The remaining reconstituted analyte should be aliquoted immediately and stored below -16 ºC for future assays (see page 4 for more details).
   b. 5 µL of the positive control can be engaged in the assay to confirm assay performance when needed

4) Preparation of MIX AlphaLISA Anti-αSMA Acceptor beads + Biotinylated Anti-αSMA Antibody:
   a. Prepare just before use.
   b. Preparation of a 10X MIX
      Run a 1/200 dilution of Anti-αSMA Acceptor beads and a 1/67 dilution of Anti-αSMA Antibody in 1X AlphaLISA Immunoassay Buffer
      For example, add 5 µL of 5 mg/mL AlphaLISA Anti-αSMA Acceptor beads and 15 µL of 500 nM Biotinylated Anti-αSMA Antibody to 980 µL of 1X AlphaLISA Immunoassay Buffer.

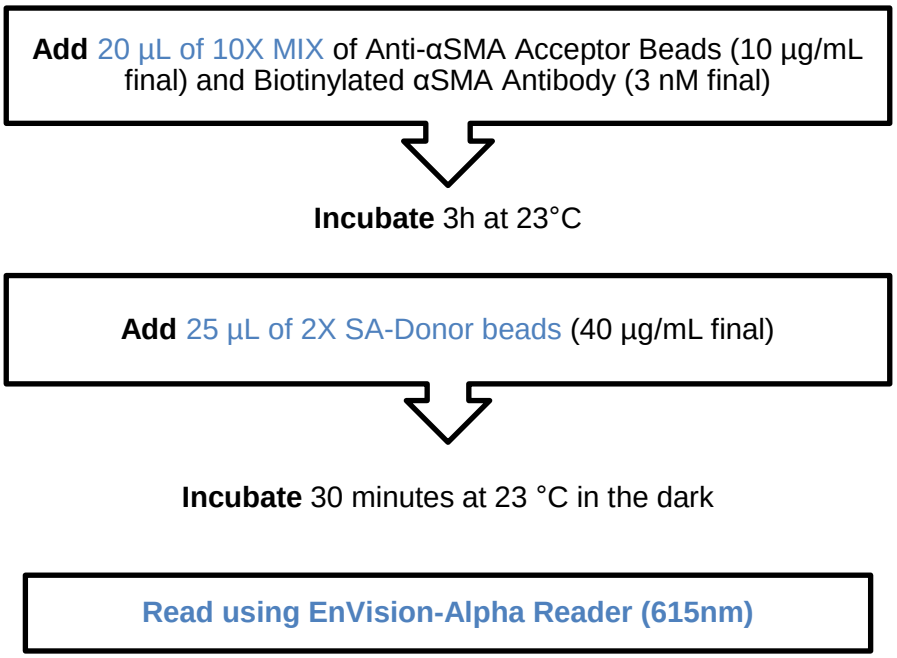
5) Preparation of Streptavidin (SA) Donor beads:
   a. Prepare just before use.
   b. Keep the beads under subdued laboratory lighting
   c. Preparation of a 2X solution
      Run a 1/63 dilution of the 5mg/mL solution of Streptavidin Donor beads in 1X AlphaLISA Immunoassay Buffer
      For example, add 20 µL of 5 mg/mL AlphaLISA Streptavidin coated Donor beads to 1230 µL of 1X AlphaLISA Immunoassay Buffer.

General Lysis Protocol:



Transfer 5 µL of lysate to 384-well plate
Dispense 5µL of the *positive control* in the same plate

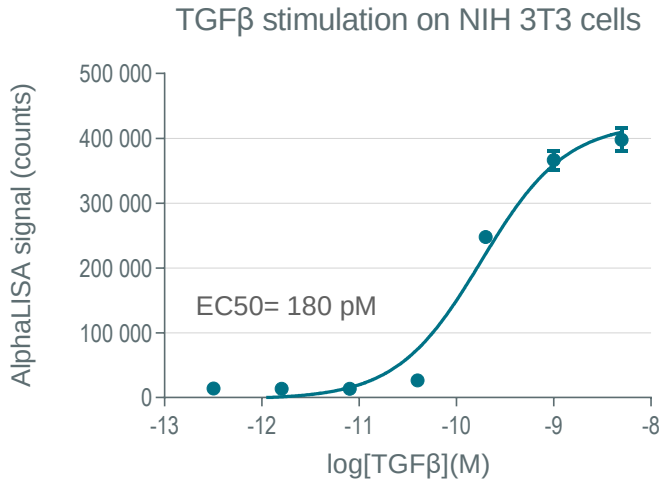
General Detection Protocol:



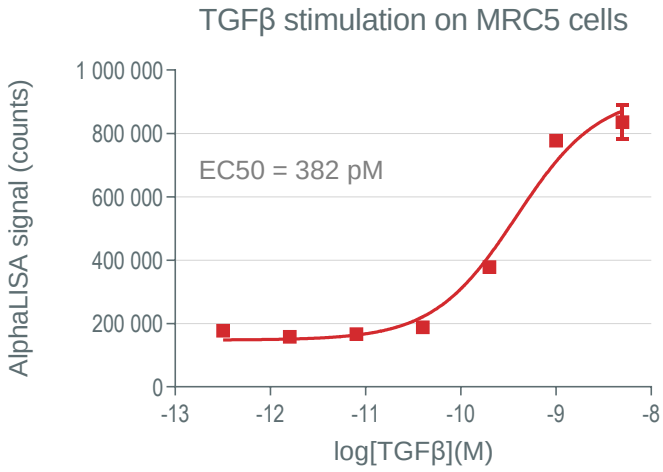
Data Analysis

- Calculate the average count value for each sample and the background wells.
- If compounds are being titrated for stimulation or inhibition, the data analysis may be done according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope).

Assay Validation



Mouse NIH3T3 cells were plated at 10,000 c/w and stimulated by serial dilution of TGFβ during 48 hours at 37°C. Supernatant was then discarded and 50µL of lysis buffer was added on top of the cells. After 30 minutes of lysis under shaking condition, 5µL of lysate were collected and transferred to a 384-well plate for detection.



Human MRC5 cells were plated at 25,000 c/w and stimulated by serial dilution of TGFβ during 24 hours at 37°C. Supernatant was then discarded and 50µL of lysis buffer was added on top of the cells. After 30 minutes of lysis under shaking condition, 5µL of lysate were collected and transferred to a 384-well plate for detection.

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Troubleshooting Guide

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

http://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/alphalisa-alphascreen-no-wash-assays/alpha-troubleshooting.html

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