TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Research Use Only. Not for use in diagnostic procedures.

Human STING WT Binding AlphaLISA Kit

Product No.: AL3144 C/F

Contents

Product Information

Application: This kit is designed to assess inhibitors of human STING WT and 2'3'cGAMP binding using a homogeneous no wash AlphaLISA assay.

Sensitivity: $IC_{50:}$ 1.14 nM (average, using 2'3'cGAMP). To calculate binding affinity using Cheng-prusoff equation use Kd_{ligand} of 80nM

Signal to background ratio: 400 using 10 nM hSTING WT protein and 80 nM biotinylated ligand

Kit contents: The kit contains 6 components: anti-6xHis Gold AlphaLISA Acceptor beads, Streptavidin-coated Donor beads, Biotinylated STING ligand, His tagged human STING WT, 2'3'cGAMP and AlphaLISA PPI buffer.

Storage: The kit components must be stored at 4 ˚C in the dark. Reconstituted reagents can be stored at –20 °C for 1 month.

Stability: This kit is stable for at least 12 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 and minimum 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 and minimum counts may vary between bead lots and the instrument used.

Analyte of Interest

Stimulator of interferon genes (STING), also known as transmembrane protein 173 (TMEM173), is a protein playing a major role in innate immunity. Upon intracellular cytosolic DNA release from pathogens such as viruses and bacteria, 2'-3'cGAMP binds to STING protein and triggers the secretion of type 1 interferon. STING Wild-Type, also called R232 is the most common variant in the human population, found at a frequency of 57.9%. Identifying new STING ligands is therefore a way to control immunity and fight autoinflammatory diseases.

Description of the AlphaLISA Assay

The AlphaLISA detection of human STING WT binding uses anti-6xHis Gold AlphaLISA® acceptor beads to capture the His tagged hSTING WT and Streptavidin-coated donor beads to capture the biotinylated ligand. Donor beads and acceptor beads come into proximity through ligand binding to hSTING WT. Excitation of the Donor beads provokes the release of singlet oxygen that triggers a cascade of energy transfer reactions in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 1).

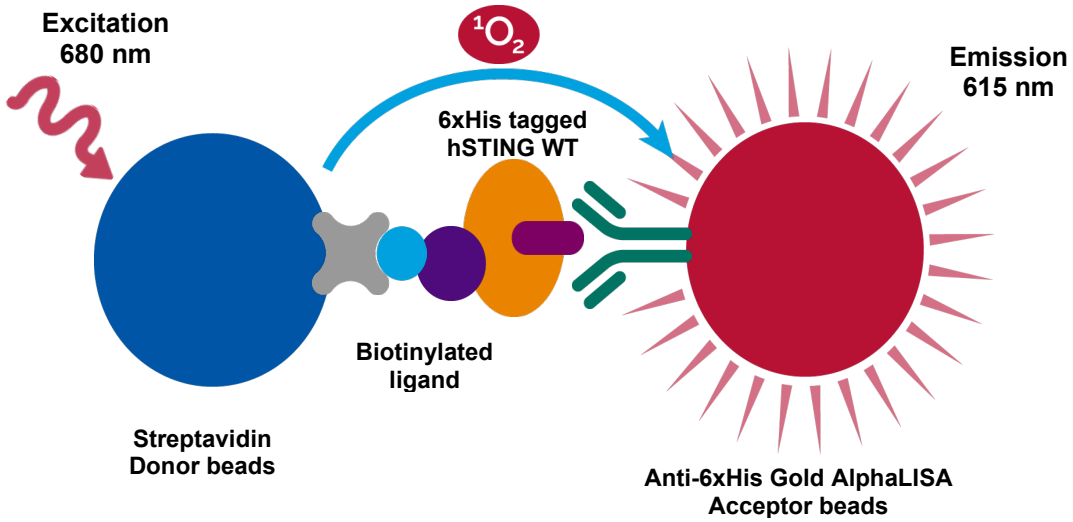


Figure 1. AlphaLISA Assay Principle.

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.
- All blood components and biological materials should be handled as potentially hazardous.

Kit Content: Reagents and Materials

Kit components	AL3144C*** (500 assay points)	AL3144F*** (5000 assay points)
Anti-6xHis Gold AlphaLISA Acceptor beads stored in PBS, 0.05% Proclin-300, pH 7.2	40 µL @ 5 mg/mL (1 brown tube, white cap)	400 µL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Proclin-300, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, black cap)	400 µL @ 5 mg/mL (1 brown tube, black cap)
Ligand (Biotinylated) stored in PBS, 0.1% Tween-20, 0.05% NaN3, pH 7.4	100µL @ 8µM (1 tube, clear cap)	1000µL @ 8µM (1 tube, clear caps)
Human STING WT protein (His tagged)	2.833 µg, lyophilized (1 tube, clear cap)	2.833 µg, lyophilized (10 tubes, clear caps)
2'3'cGAMP Standard	6.74 µg, lyophilized (1 tube, clear cap)	6.74 µg, lyophilized (1 tube, clear cap)
AlphaLISA PPI Buffer (5X)	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute 2'3'cGAMP and hSTING WT protein in 100 µL Milli-Q® grade H_2O respectively. The reconstituted reagents should be used within 60 minutes. After reconstitution, aliquot and store unused protein at -20 °C for 1 month. Avoid multiple freeze-thaw cycles.

** Extra buffer can be ordered separately (cat # AL015C: 10 mL, cat # AL015F: 100 mL).

*** The number of assay points is based on an assay volume of 20 µL in 384 well 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.

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Adhesive Sealing Film	PerkinElmer Inc.	6050185
AlphaPlate-384, Shallow Well (ProxiPlate)	PerkinElmer Inc.	6008350 6008359
EnVision®-Alpha Reader	PerkinElmer Inc.	-

The following reagents might be required for particular applications:

Item	Supplier	Catalog number
2'3'cGAMP Standard	PerkinElmer Inc.	AL3144S
Cyclic di-AMP sodium salt	Sigma Aldrich	SML1223
Cyclic di-GMP sodium salt	Sigma Aldrich	SML1228
DMXAA	Sigma Aldrich	D5817

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 pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend the beads by vortexing before use. Do not vortex the proteins.
- Use Milli-Q® grade H_2O to dilute 5X AlphaLISA PPI Buffer and to reconstitute the lyophilized 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 Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film.
- 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, 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.

Competition Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an ***example*** for generating 250 assay points in a 20 µL final assay volume per point. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes. If a different number of samples are tested, the volumes of all reagents must be adjusted accordingly.
- The dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

One Incubation Step Protocol described as below:

1) Preparation of 1X AlphaLISA PPI Buffer (for 10 mL):

   Add 2 mL of 5X AlphaLISA PPI Buffer to 8 mL H_2O.

2) Preparation of serial dilutions of 2'3'cGAMP in 1X AlphaLISA PPI buffer as follows:

   a. Reconstitute lyophilized 2'3'cGAMP (6.74 µg) in 100 µL H_2O to make 100µM of 2'3'cGAMP.
   b. Prepare serial dilutions of 4X 2'3'cGAMP in 1x AlphaLISA PPI buffer as follows, change tips between each dilution:

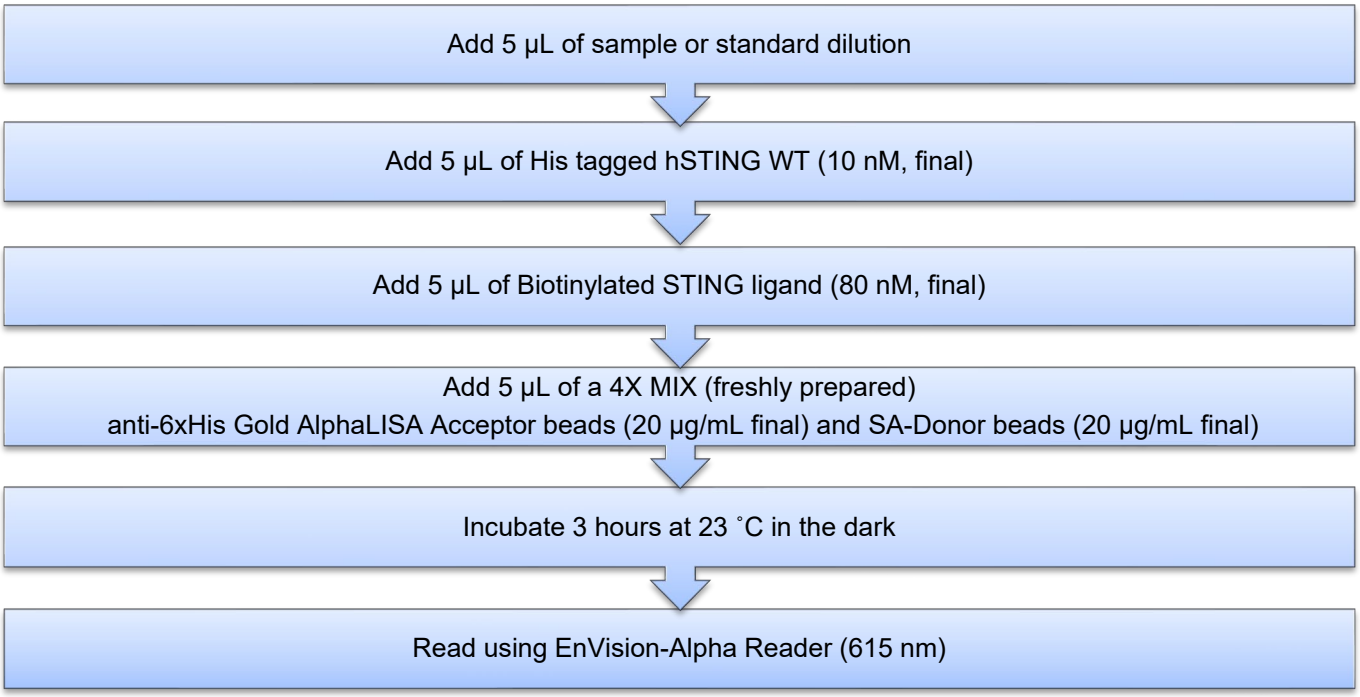
Tube	Volume of Antibody	Volume of 1X buffer	[Standard] (nM) (4X)	[Standard] (nM) (1X)
A	8 µL of 8µM stock	192 µL	4000	1000
B	60 µL of tube A	140 µL	1 200	300
C	60 µL of tube B	120 µL	400	100
D	60 µL of tube C	140 µL	120	30
E	60 µL of tube D	120 µL	40	10
F	60 µL of tube E	140 µL	12	3
G	60 µL of tube F	120 µL	4	1
H	60 µL of tube G	140 µL	1	0.3
I	60 µL of tube H	120 µL	0.4	0.10
J	60 µL of tube I	140 µL	0.12	0.03
K	60 µL of tube J	120 µL	0.04	0.01
L	0	140 µL	0	0

3) Preparation of 4X His tagged hSTING WT (40 nM):
   a. Reconstitute lyophilized hSTING WT (2.833 µg) in 100 µL H_2O to make 1µM hSTING WT.
   b. Add 50 µL of 1µM hSTING WT to 1200 µL 1X AlphaLISA PPI buffer.
   c. Prepare just before use.

4) Preparation of 4X biotinylated STING ligand (320 nM):
   a. Add 50 µL of 8µM biotinylated STING ligand to 1200 µL 1X AlphaLISA PPI buffer.
   b. Prepare just before use.

5) Preparation of the mix of 4X Anti-6xHis Gold AlphaLISA Acceptor beads (80 µg/mL) and 4X Streptavidin (SA) Donor beads (80 µg/mL):
   a. Keep the beads under subdued laboratory lighting.
   b. Add 20µL of 5 mg/mL Anti-6xHis Gold AlphaLISA Acceptor beads and 20µL of 5 mg/mL SA-Donor beads to 1210 µL of 1X AlphaLISA PPI buffer
   c. Prepare just before use.

6) In a shallow well AlphaPlate (384 wells):



Read Settings: 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).

Typical competitive binding Data:

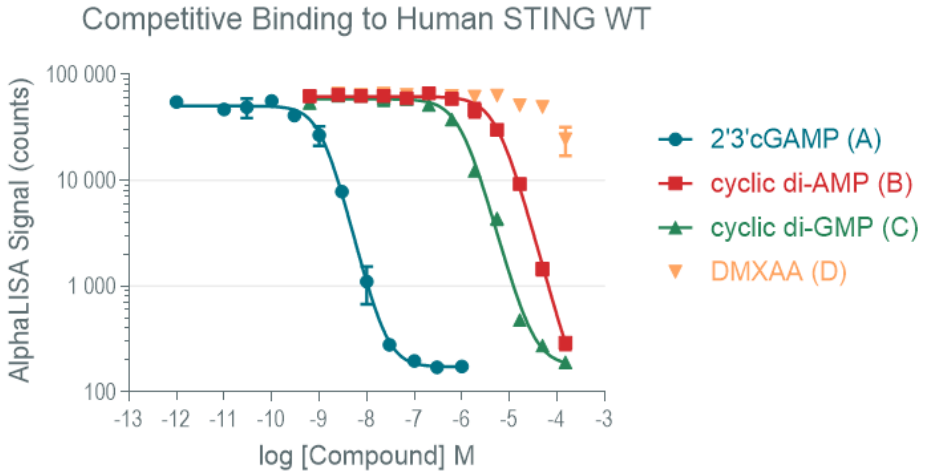


Figure 2. Competitive Binding: 2'3'cGAMP (A), cyclic di-AMP (B) and cyclic di-GMP (C) competitively binds to hSTING WT with IC_{50} =1.14nM, 5.44µM and 0.96µM respectively. DMXAA (D) was measured as negative control. All IC_{50} values were calculated by using nonlinear regression fitting with GraphPad Prism 7.

Troubleshooting Guide

You will find below recommendations for common situations that you might encounter with your AlphaLISA binding assay. If further assistance is needed, do not hesitate to contact our technical support team for assistance.

Issue	Recommendations and Comments
High background signal	• Buffer is not freshly made. Make new. • Incubation time is longer than recommended range.
Low AlphaLISA signal	• Optimize EnVision with Plate format.
High variation between replicates or low Z' values	• Make sure that reagents are at the bottom of the well by tapping or swirling the plate gently on a smooth surface after each addition.

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

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

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