Description

The FGL1:LAG3 Inhibitor Screening Assay is an AlphaLISA®-based assay designed to measure the inhibition of LAG3 binding to FGL1 in a homogeneous 384-reaction format. The FGL1:LAG3 Inhibitor Screening Assay Kit contains sufficient amounts of purified recombinant FGL1 and LAG3 proteins, and assay buffer for 400 reactions. The assay is straightforward: first, FGL1 and LAG3 are incubated with or without a compound of interest. Then, acceptor and donor beads are added to the reaction, followed by reading the Alpha-counts.

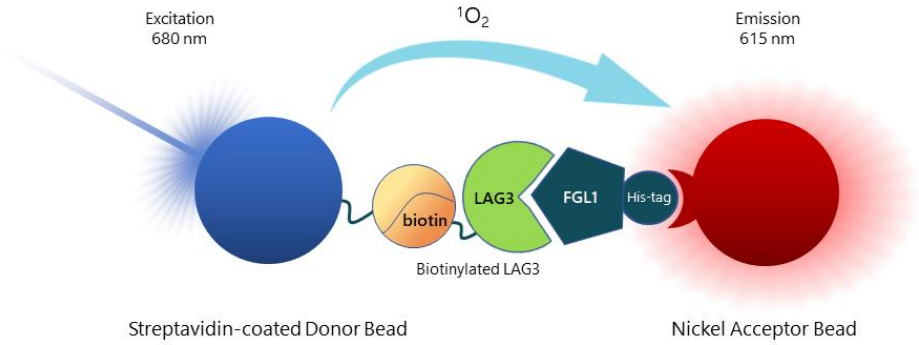

Illustration of the assay principle.

FGL1 contains a His tag recognized by the Nickel chelate acceptor bead, whereas LAG3 is biotinylated, allowing its binding to the streptavidin-coated acceptor bead. Interaction between FGL1 and its receptor LAG3 brings the donor and acceptor beads in proximity. A singlet oxygen generated upon excitation of the donor bead leads to the excitation of the acceptor bead, which emits light. Light emission in the assay is proportional to the level of interaction. AlphaLISA™ immunoassays are a no-wash alternative to ELISA. These assays are robust, highly amenable to high throughput applications, and ideal for a minimal hands-on approach.

Background

Lymphocyte-activation gene 3 (LAG3, also known as CD223) is a cell surface receptor that negatively regulates the activation and proliferation of T cells. Fibrinogen-like protein 1 (FGL1), a liver-secreted protein present in the plasma, is a functional LAG3 ligand. Blockade of the FGL1-LAG3 interaction has been proposed as a therapeutic strategy to promoting antitumor responses in cancer patients.

Applications

Screen for inhibitors of LAG3 binding to FGL1.

Supplied Materials

Catalog #	Name	Amount	Storage
	FGL1, His Tag	10 µg	-80°C
71147	LAG3 (CD223), Biotin-Labeled (Human) HiP™	32 µg	-80°C
79311	3x Immuno Buffer	4 ml	-20°C

Materials Required but Not Supplied

- AlphaLISA® Nickel Chelate acceptor beads, 5 mg/ml PerkinElmer #AL108C
- AlphaScreen® Streptavidin donor beads, 5 mg/ml PerkinElmer #6760002S
- Optiplate - 384 PerkinElmer #6007290
- AlphaScreen® microplate reader
- Adjustable micropipettor and sterile tips

Stability

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

Green and blue dyes, such as Trypan Blue, absorb light in the AlphaScreen™ signal emission range (520-620 nm). Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN3) or metal ions (Fe2+, Fe3+, Cu2+, Zn2+ and Ni2+). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen™ assays.

Assay Protocols

All samples and controls should be tested in duplicate.

1. Thaw **FGL1-His** and **LAG3-Biotin** on ice. Briefly spin the tubes containing the proteins to recover their full contents. If the assay plate is going to be used more than once, prepare enough protein for this portion of the assay and aliquot the remaining undiluted protein into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

   *Note: **FGL1-His** and **LAG3-Biotin** are very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not reuse the diluted proteins.*

2. Dilute one-part **3x Immuno Buffer** with 2 parts of distilled water (3-fold dilution) to make **1x Immuno Buffer**. Prepare only a sufficient quantity needed for the assay; store the remaining stock buffer in aliquots at -20°C.

3. Dilute **LAG3-Biotin** in **1x Immuno Buffer** to 20 ng/µl. Keep the diluted protein on ice until use. Discard the remaining diluted protein after use.

4. Add 4 µl of **1x Immuno Buffer** to wells designated “Blank”.

5. Add 4 µl of diluted **LAG3-Biotin** to wells designated “Test Inhibitor” and “Positive Control”.

6. Prepare the Test Inhibitor (2 µl/well): For a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 10 µl.

Without DMSO

a. If the Test Inhibitor is water-soluble, prepare serial dilutions of the compound in **1x Immuno Buffer**, 5-fold more concentrated than the desired final concentrations. For the positive and negative controls, use **1x Immuno Buffer** (Diluent Solution).

Or

With DMSO

a. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO (*i.e.*, if the highest testing concentration is 50 µM, prepare a 5 mM solution in 100% DMSO). Then dilute the inhibitor 20-fold in **1x Immuno Buffer** to prepare the highest concentration of the 5-fold intermediate solution (i.e., to test at 50 µM, prepare a 250 µM intermediate solution by adding 5 µl of 5 mM inhibitor solution to 95 µl of **1x Immuno Buffer**). The concentration of DMSO is now 5%.
b. Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in **1x Immuno Buffer** to keep the concentration of DMSO constant.
c. For positive and negative controls, prepare 5% DMSO in **1x Immuno Buffer** (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO in the reaction should not exceed 1%.

7. Add 2 µl of 5-fold intermediate serial dilutions of the Test Inhibitor to the testing wells.

8. Add 2 µl of Diluent Solution (for example 5% DMSO in **1x Immuno Buffer)** to the "Positive control" and "Negative control" wells.

9. Dilute **FGL1-His** in **1x Immuno Buffer** to 6 ng/µl. Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use.

10. Add 4 µl of diluted **FGL1-His** to all wells. Incubate the plate at room temperature for 1 hour.

Component	Positive Control	Blank	Test Inhibitor
FGL1-His (6 ng/µl)	4 µl	4 µl	4 µl
1x Immuno Buffer	-	4 µl	-
Test Inhibitor	-	-	2 µl
Diluent Solution	2 µl	2 µl	-
LAG3-Biotin (20 ng/µl)	4 µl	-	4 µl
Total	**10 µl**	**10 µl**	**10 µl**

11. Dilute the Nickel Chelate Acceptor beads (PerkinElmer #AL108C) and the Streptavidin Donor beads (PerkinElmer #6760002S) at 1:500 and 1:250 respectively in **1x Immuno Buffer** (i.e., for 400 reactions, ~8 ml of the detection reagent is needed. Therefore add 16 µl of Nickel Chelate Acceptor beads and 32 µl of Streptavidin beads to 8 ml of **1x Immuno Buffer**).

12. Add 20 µl of acceptor/donor beads mix to all the wells. Incubate 30 minutes at room temperature.

13. Read Alpha-counts using a compatible plate reader.

Example of Assay Results:

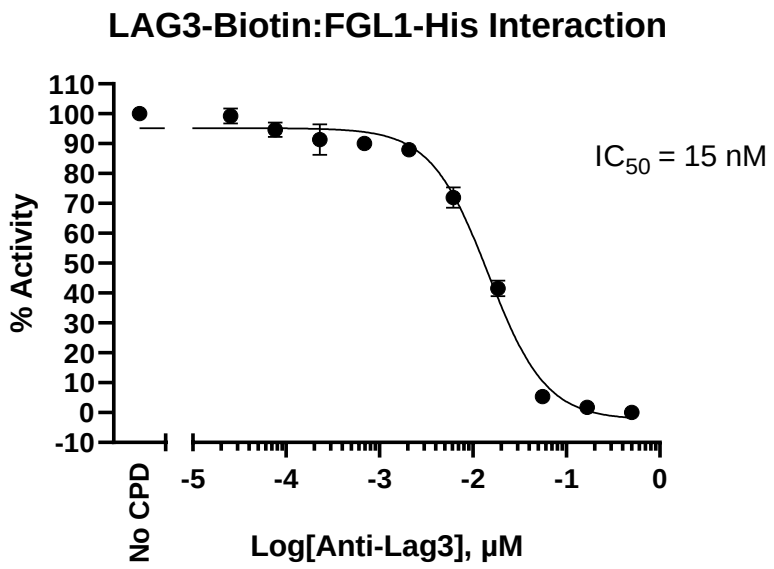

Figure 1: Inhibition of FGL1:LAG3 interaction using a neutralizing anti-LAG3 antibody.
Inhibition of FGL1:LAG3 binding was evaluated in the presence of increasing concentrations of anti-LAG3 Neutralizing Antibody (BPS Bioscience #71219) using FGL1:LAG3 Inhibitor Screening Assay Kit (BPS Bioscience #78824).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References

1. Wang J, *et al.* Fibrinogen-like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3. *Cell* 2019; **176(1-2)**: 334-347.
2. Visan I. New ligand for LAG-3. *Nature Immunol.* 2019; **20(2)**: 111.

Related Products

Products	*Catalog #*	*Size*
FGL1:LAG3 TR-FRET Assay Kit	79739	384 reactions
PE Labeled Anti-LAG3 Antibody	71226	Various
LAG3 / NFAT Reporter Jurkat Recombinant Cell Line	71278	2 vials
LAG3 (CD223), Fc Fusion (Human)	71146	100 µg
LAG3 (CD223), Fc Fusion (Mouse)	79050	100 µg