Description

The LAG3:FGL1[Biotinylated] Inhibitor Screening Assay Kit is designed to measure the binding of LAG3 (lymphocyte-activation gene 3) to FGL1 (fibrinogen-like protein 1) for screening and profiling applications. The LAG3:FGL1[Biotinylated] Inhibitor Screening Assay Kit comes in a convenient 96-well format, with enough recombinant purified biotinylated FGL1, LAG3 (amino acids 23-450), blocking and assay buffer and detection reagents for 100 enzyme reactions.

Background

LAG3 (lymphocyte-activation gene 3), also known as CD223, is a cell surface receptor that functions as an inhibitory immune checkpoint. It is found in $CD4^+$ and $CD8^+$ T cells, NK (natural killer) cells, NKT cells and Treg (T regulatory) cells. LAG3 is involved in T cell exhaustion and proliferation. It has several ligands, which include galectin-3, α-synuclein and Fibrinogen-like protein 1 (FGL1). LAG3 and FGL1 are found in tumor cells, where they can serve as immune checkpoints and potentially as oncogenes, and their levels correlate with patient prognosis. LAG3/FGL1 plays a role in immune cell function, cytokine production and tumor growth and metastasis. Several monoclonal antibodies targeting LAG3 are currently being tested, such as relatlimab, and have shown promising results alone or in combination therapy. Blockade of the FGL1-LAG3 interaction can promote antitumor immunity and may benefit patients that have developed tumor immune resistance. For instance, high levels of FGL1 may contribute to gefitinib therapy resistance while high LAG3 levels were found in patients after EGFR (epidermal growth factor receptor)-TKI (tyrosine kinase inhibitor) treatment. A deeper understanding of the importance of this complex in health and disease will allow the development of new cancer therapy strategies.

Applications

Screening of small molecules and antibodies that inhibit binding of LAG3 to FGL1.

Supplied Materials

Catalog #	Name	Amount	Storage
71229	LAG3, His-Tag*	50 µg	-80°C
100327	FGL1, Fc-Fusion (IgG1), Avi-Tag, Biotin-Labeled	5 µg	-80°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	96-well white nickel coated microplate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1x PBS (phosphate buffer saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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Storage Conditions

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

This kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control", "Ligand Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Anti-LAG3 antibody (#71219) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1: Coat plate

1. Thaw **Blocking Buffer 2** enzyme on ice.
2. Add 50 µl of Blocking Buffer 2 to each well, except "Ligand Control" wells.
3. Incubate at 4°C overnight.
4. Tap the plate a onto clean paper towel to remove the liquid.
5. Wash the plate three times using 100 µl of PBST per well.
6. Tap the plate onto a clean paper towel to remove the liquid.
7. Thaw **LAG3** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.
8. Dilute LAG3 to **8 µg/ml** with PBST (50 µl/well, except "Ligand Control").
9. Add 50 µl of diluted LAG3 to each well, except "Ligand Control".
10. Incubate at Room Temperature (RT) for 3 hours with slow agitation.

11. Dilute 3-fold the 3x Immuno Buffer 1 with distilled water. This makes 1x Immuno Buffer 1.

    Note: 30 ml of 1x Immuno Buffer 1 are enough for a 96-well plate. 3x Immuno Buffer 1 is necessary for other steps in the protocol.

12. Tap the plate onto a clean paper towel to remove the liquid.

13. Wash plate three times with 100 µl/well of 1x Immuno Buffer 1.

14. Tap the plate onto a clean paper towel to remove the liquid.

15. Block the wells by adding 100 µl of Blocking Buffer 2 to every well.

16. Incubate for 15 minutes at RT with slow agitation.

17. Tap the plate onto a clean paper towel to remove the liquid.

18. Wash plate three times with 100 µl/well of 1x Immuno Buffer 1.

19. Tap the plate onto a clean paper towel to remove the liquid.

Step 2: Reaction

1. Prepare a Master Mix (25 µl/well): N wells x (10 µl of 3x Immuno Buffer 1 + 15 µl of distilled water).

2. Add 25 µl of Master Mix to every well.

3. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration in 1x Immuno Buffer 1.

   For the positive and negative controls, use 1x Immuno Buffer 1 (Diluent Solution).

   OR

   3.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Immuno Buffer 1 (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Immuno Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Immuno Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of Test Inhibitor to each well labeled as “Test Inhibitor”.
5. Add 5 µl of Diluent Solution to the “Positive Control”, “Ligand Control” and “Blank” wells.
6. Incubate at RT for 1 hour with slow agitation.
7. Thaw **FGL1[Biotin]** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.
8. Dilute FGL1 to **0.25 ng/µl** with 1x Immuno Buffer 1 (20 µl/well).
9. Initiate the reaction by adding 20 µl of diluted FGL1 to the wells designated “Positive Control”, “Ligand Control” and "Test Inhibitor."
10. Add 20 µl of 1x Immuno Buffer 1 to the “Blank” wells.
11. Incubate at RT for 1 hour with slow agitation.

	Blank	**Ligand Control (uncoated)**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl	25 µl
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
Diluted FGL-1 (0.25 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 and tap the plate onto a clean paper towel.
13. Block the wells by adding 100 µl of Blocking Buffer 2 to every well.
14. Incubate for 15 minutes at RT with slow agitation.
15. Tap the plate onto clean paper towel to remove the liquid.
16. Wash plate three times with 100 µl/well of 1x Immuno Buffer 1.
17. Tap the plate onto a clean paper towel to remove the liquid.

Step 3: Detection

1. Dilute Streptavidin-HRP 1000-fold in Blocking Buffer 2 (100 µl/well).
2. Add 100 µl of diluted Streptavidin-HRP to each well.
3. Incubate for 1 hour at RT with gentle agitation.
4. Wash three times with 100 µl of 1x Immuno Buffer 1 and tap the plate onto clean paper towel.
5. Block the wells by adding 100 µl of Blocking Buffer 2 to every well.
6. Incubate at RT for 15 minutes.
7. Tap the plate onto a clean paper towel to remove the liquid.
8. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
9. Add 100 µl of mix per well.
10. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
11. The "Blank" value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

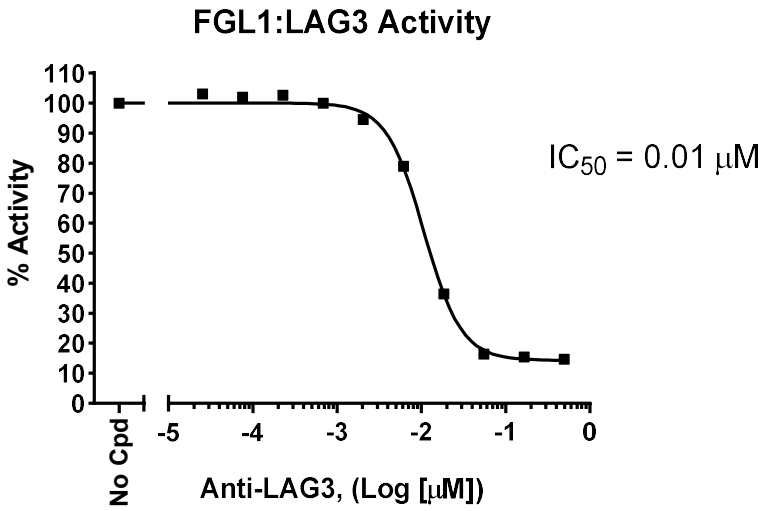

Figure 1: Inhibition of binding of LAG3:FGL1 by anti-LAG3 antibody.
A plate was coated with LAG3, followed by incubation with FGL1 in the presence of increasing concentrations of Anti-LAG3 Neutralizing Antibody (#79789). Luminescence was measured using a Bio-Tek microplate reader.

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

References

Wang J., *et al.,* 2019 *Immunol.* 156.1: 74-85
Xu W., *et al.*, 2018 *Cell. Mol. Immunol.* 15(5): 438
Shi A., *et al.*, 2022 *Front Immunol.* 12:785091

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
FGL1:LAG3 TR-FRET Assay Kit	79739	96/384 reactions
LAG3/ IL-2 reporter - Jurkat Recombinant Cell Line	79813	2 vials
LAG3/ NFAT reporter - Jurkat Recombinant Cell Line	71278	2 vials
LAG3 CRISPR/Cas9 Lentivirus (Non-Integrating)	78060	500 µl x 2
LAG3 CRISPR/Cas9 Lentivirus (Integrating)	78053	500 µl x 2
LAG3 (CD223), Fc-Fusion, Avi-Tag, Biotin-Labeled (Human) Recombinant	71147	25 µg/50 µg

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