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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data sheet

LAG3:FGL1[Biotinylated] Inhibitor Screening Assay Kit

Catalog #79789
Size: 96 reactions

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, is a functional LAG3 ligand. Blockade of the FGL1-LAG3 interaction is implicated in promoting antitumor immunity.

DESCRIPTION: The *LAG3:FGL1[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of LAG3:FGL1. This kit comes in a convenient 96-well format, with biotin-labeled FGL1, purified His-tagged LAG3, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled FGL1 by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, LAG3 is attached on a nickel-coated 96-well plate. Next, biotinylated FGL1 is incubated with LAG3 on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71229	LAG3, His-Tag, HiP™	50 µg	-80 °C	Avoid multiple freeze/thaw cycles!
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	15 µl	-20 °C	
79670	ELISA ECL Substrate A	6 ml	Room Temp.	
	ELISA ECL Substrate B	6 ml	Room Temp.	
	96-well white nickel coated microplate	1	+4 °C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

APPLICATIONS: This kit is useful for screening for inhibitors of LAG3 binding to FGL1.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

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REFERENCES:

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

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Coating the plate with LAG3-His:

1) Thaw **LAG3-His** on ice. Upon first thaw, briefly spin tube containing **LAG3-His** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **LAG3-His** in aliquots at -80°C. Note: **LAG3-His** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **LAG3-His** to 8 µg/ml in PBS.
3) Add 50 µl of diluted **LAG3-His** solution to each well and incubate for 1 hour at room temperature with slow shaking. Leave a couple of wells empty (uncoated), for use with the "Ligand Control" (see below).
4) Dilute **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with water. (Keep a portion of the **3x Immuno Buffer 1** undiluted, for use in Step 1 below).
5) Decant to remove supernatant. Wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
6) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature with slow shaking. Remove supernatant as described in 5).

Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1** + 15 µl distilled water)
2) Add 25 µl of master mixture to each well. Use uncoated wells for the "Ligand Control".
3) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Ligand Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer). Incubate at room temperature for one hour with slow shaking.
4) Thaw **FGL1-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **FGL1-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80 °C. Note: ***FGL1-biotin*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
5) Dilute **FGL1-biotin** to 1.5 ng/µl (25 nM) in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.
6) Add 20 µl of **1x Immuno Buffer 1** to the well designated "Blank".

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	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer 1	10 µl	10 µl	10 µl	10 µl
Distilled water	15 µl	15 µl	15 µl	15 µl
Test Inhibitor	-	-	-	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
FGL1-biotin (1.5 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

7) Initiate reaction by adding 20 µl of diluted **FGL1-biotin** (see Step 1-5) to wells labeled "Positive Control", "Ligand Control" and "Test Inhibitor". Incubate at room temperature for one hour with slow shaking.
8) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
9) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-8.

Step 2:

1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2**.
2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.
3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towel to remove liquid.
4) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.
5) Just before use, mix on ice 50 µl **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B**, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.
6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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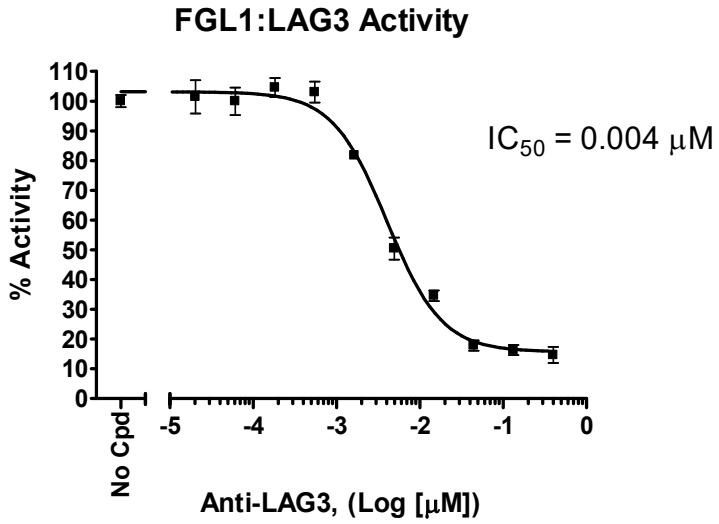
Reading Chemiluminescence:

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 milliseconds. 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 of Assay Results:



Inhibition of LAG3:FGL1 binding using Anti-LAG3, Neutralizing antibody (BPS Bioscience #71219), in the LAG3:FGL1[Biotinylated] Inhibitor Screening Assay Kit (BPS Bioscience #79789). Luminescence was measured using a Bio-Tek fluorescent microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

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RELATED PRODUCTS:

Product Name	Catalog#	Size
Anti-LAG3, Neutralizing Antibody	71219	100 µg
PE labeled anti-LAG3 antibody	71226-1	50 µg
PE labeled anti-LAG3 antibody	71226-2	100 µg
FGL1:LAG3 TR-FRET Assay Kit	79739-1	96 rxns
FGL1:LAG3 TR-FRET Assay Kit	79739-2	384 rxns
LAG3 / NFAT Reporter - Jurkat Recombinant Cell Line	71278	2 vials
LAG3 (CD223), Fc fusion (Human)	71146	100 µg
LAG3 (CD223), Biotin-labeled (Human) HiP™	71147	50 µg
LAG3 (CD223), Fc fusion (Mouse)	79050	100 µg
LAG3 (CD223), Biotin-labeled (Mouse) HiP™	79003	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	LAG3 or FGL1-biotin has lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh FGL1-biotin, (BPS Bioscience #100327) and fresh LAG3 (BPS Bioscience #71229). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in PBST
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of FGL1-biotin (BPS Bioscience #100327) to create a standard curve