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Data sheet
FGL1:LAG3 TR-FRET Assay Kit
Catalog #79739-2
Size: 384 reactions

BACKGROUND: Lymphocyte-activation gene 3 (LAG3, also CD223) is a cell surface receptor that negatively regulates 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 FGL1:LAG3 TR-FRET Assay is designed to measure the inhibition of LAG3 binding to FGL1 in a homogeneous 384 reaction format. This TR-FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; a sample containing biotinylated LAG3, His-tagged FGL1 protein, and an inhibitor are incubated for one hour. Then, anti-His Tb donor and dye-labeled acceptor are added and fluorescence intensity is measured using a fluorescence reader

COMPONENTS:

Catalog #	Component	Amount	Storage	
100330	FGL1, His tag	2 x 5 µg	-80°C	Avoid multiple freeze/thaw cycles!
71147	LAG3 (CD223), Biotin-labeled (Human) HiP™	2 x 10 µg	-80°C	
30017	Anti-His Tb Donor	2 x 10 µl	-20°C	
	Dye-labeled Acceptor	2 x 10 µl	-20°C	
	3x FGL1 TR-FRET Buffer	4 ml	-20°C	
	White, 384-well microtiter plate	1 unit	Room. temp	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluroescence microplate reader capable of measuring Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET)
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.

REFERENCES:
Wang, J., *et al. Cell* 2019, **176(1-2)**: 334-347
Visan. I., *et al. Nature Immunol.* 2019, **20(2)**: 111

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

Step 1:

1) Thaw **FGL1-His** on ice. Upon first thaw, briefly spin tube containing the protein to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C. Note: **FGL1-His** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute one part **3x FGL1 TR-FRET Buffer** with 2 parts of distilled water (3-fold dilution) to make **1x FGL1 TR-FRET Buffer**. Prepare only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.
3) Dilute **FGL1-His** in **1x FGL1 TR-FRET Buffer** to 5 ng/µl (50 nM final assay concentration). Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use.
4) Dilute **LAG3-Biotin** in **1x FGL1 TR-FRET Buffer** to 10 ng/µl (50 nM final assay concentration). Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use.
5) Add 4 µl of diluted **FGL1-His** to all wells.
6) Dilute test inhibitor into **1x FGL1 TR-FRET Buffer**. Add 2 µl of test inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control" and "Blank", add 2 µl of the same solution without inhibitor (**1x FGL1 TR-FRET Buffer** with the same concentration of DMSO as in the test inhibitor solution).
7) Add 4 µl of **1x FGL1 TR-FRET Buffer** to wells designated "Blank".
8) Add 4 µl of diluted **LAG3-Biotin** to wells designated "Test Inhibitor" and "Positive Control". Incubate the plate at room temperature for 1 hour.

	Positive Control	**Blank**	**Test Inhibitor**
FGL1-His (5 ng/µl)	4 µl	4 µl	4 µl
1x FGL1 TR-FRET Buffer	-	4 µl	-
Test Inhibitor	-	-	2 µl
Inhibitor buffer	2 µl	2 µl	-
LAG3-Biotin (10 ng/µl)	4 µl	-	4 µl
Total	**10 µl**	**10 µl**	**10 µl**

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Step 2:

1) Dilute **Anti-His Tb Donor** 100-fold with **1x FGL1 TR-FRET Buffer**. Add 5 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

1) Dilute **Dye-labeled Acceptor** 100-fold with **1x FGL1 TR-FRET Buffer**. Add 5 µl per well. Incubate at room temperature for 60 minutes.
2) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings:

Reading Mode	Value
Excitation Wavelength	320 ± 10 nm
Emission Wavelength	620 ± 10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	320 ± 10 nm
Emission Wavelength	665 ± 10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:

Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TRFRET ratio (665 nm emission/620 nm emission). If desired, data can be normalized to percent inhibition. Typically for inhibitor screens, the TR-FRET value from the positive control is set to zero percent inhibition and the TR-FRET value from the negative control is set to one hundred percent inhibition.

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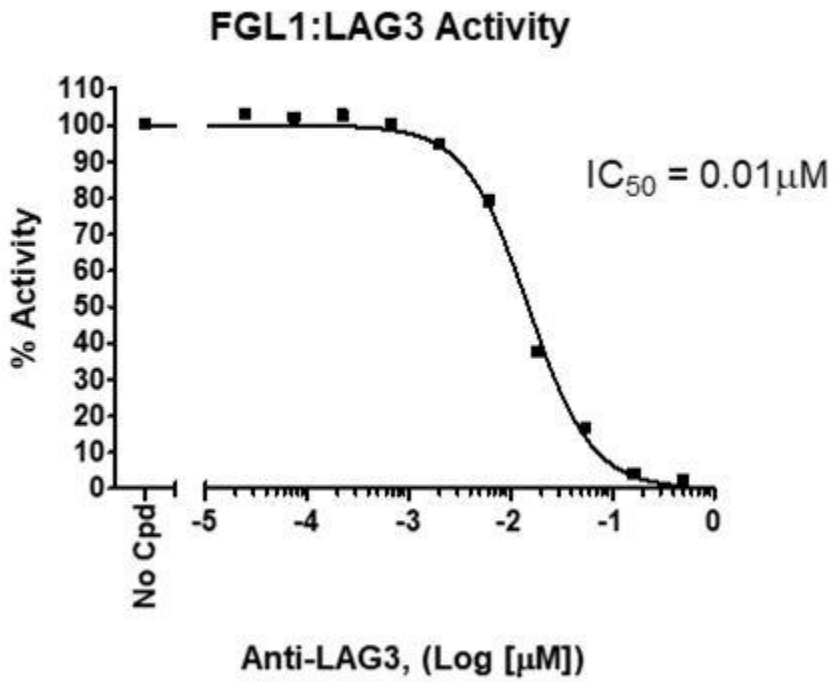
Example of assay results:



Inhibition of FGL1:LAG3 binding using the LAG3 Neutralizing Antibody, BPS Bioscience #71219 and the *FGL1:LAG3 TR-FRET Assay Kit (#79739)*. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

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 Homogeneous Assay Kit	xxxxx	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