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Data sheet
FGL1:LAG3 TR-FRET Assay Kit
Catalog #79739-1
Size: 96 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 96 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 | 5 µg | -80°C | Avoid multiple freeze/thaw cycles! |
| 71147 | LAG3 (CD223), Biotin-labeled (Human) HiP™ | 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 | |
| 79696 | White, 96-well microtiter plate | 1 | Room temp | |

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescence 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 $5\text{ ng}/\mu\text{l}$ (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 $10\text{ ng}/\mu\text{l}$ (50 nM final assay concentration). Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use.
- 5) Add $10\text{ }\mu\text{l}$ of diluted **FGL1-His** to all wells.
- 6) Dilute test inhibitor into **1x FGL1 TR-FRET Buffer**. Add $5\text{ }\mu\text{l}$ of test inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control" and "Blank", add $5\text{ }\mu\text{l}$ 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 $10\text{ }\mu\text{l}$ of **1x FGL1 TR-FRET Buffer** to wells designated "Blank".
- 8) Add $10\text{ }\mu\text{l}$ 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 ($5\text{ ng}/\mu\text{l}$) | $10\text{ }\mu\text{l}$ | $10\text{ }\mu\text{l}$ | $10\text{ }\mu\text{l}$ |
| 1x FGL1 TR-FRET Buffer | - | $10\text{ }\mu\text{l}$ | - |
| Test Inhibitor | - | - | $5\text{ }\mu\text{l}$ |
| Inhibitor buffer | $5\text{ }\mu\text{l}$ | $5\text{ }\mu\text{l}$ | - |
| LAG3-Biotin ($10\text{ ng}/\mu\text{l}$) | $10\text{ }\mu\text{l}$ | - | $10\text{ }\mu\text{l}$ |
| Total | $25\text{ }\mu\text{l}$ | $25\text{ }\mu\text{l}$ | $25\text{ }\mu\text{l}$ |

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Step 2:

- 1) Dilute **Anti-His Tb Donor** 100-fold with **1x FGL1 TR-FRET Buffer**. Add 12.5 μ l 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 12.5 μ l 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 \pm 10 nm |
| Emission Wavelength | 320 \pm 10 nm |
| Lag Time | 60 μ s |
| Integration Time | 500 μ s |
| Excitation Wavelength | 320 \pm 10 nm |
| Emission Wavelength | 665 \pm 10 nm |
| Lag Time | 60 μ s |
| Integration Time | 500 μ s |

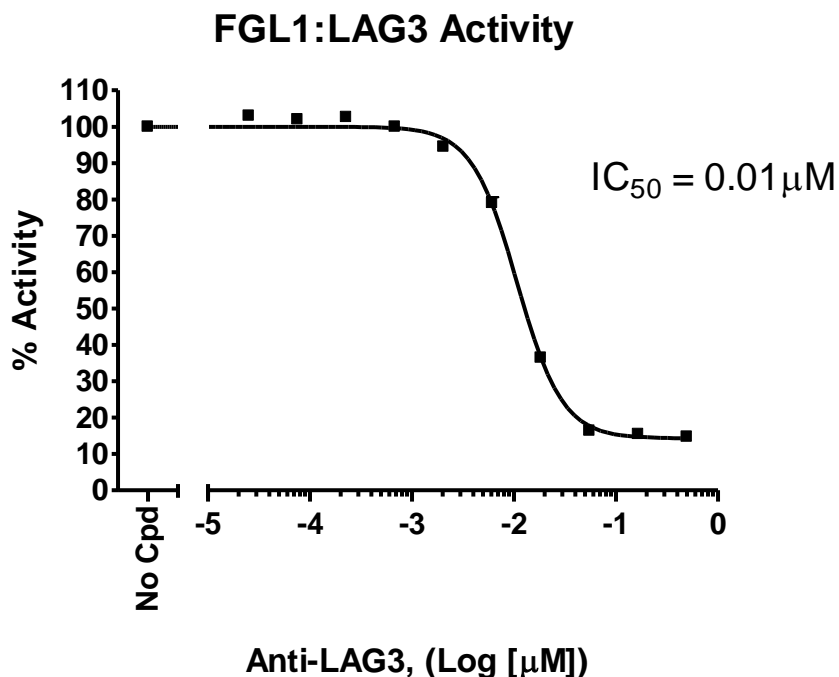
CALCULATING RESULTS:

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* (#79739-1). Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

RELATED PRODUCTS:

| <u>Product Name</u> | <u>Catalog#</u> | <u>Size</u> |
|---|-----------------|-------------|
| FGL1:LAG3 TR-FRET Assay Kit | 79739 | 384 rxns |
| 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 |
| 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 |

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