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Data Sheet
GITR:GITRL TR-FRET Assay Kit
Catalog # 79054

DESCRIPTION:
The GITR:GITRL TR-FRET Assay is designed to measure the inhibition of GITR binding to GITRL in a homogeneous 384 reaction format. This 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 GITR, GITRL, anti-His Tb donor, dye-labeled acceptor, and an inhibitor is incubated for two hours. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71256	GITR, biotinylated	15 µg	-80°C	***(Avoid freeze/thaw cycles!)***
71190	GITRL-His	2 µg	-80°C	
	Anti-His Tb Donor	2 x 10 µl	-20°C	
	Dye-labeled Acceptor	2 x 10 µl	-20°C	
	3x GITR TR FRET Assay Buffer	4 ml	-20°C	
	White, non-binding, low volume, 384-well microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescence microplate reader capable of measuring Time Resolved Fluorescence ResonanceEnergy Transfer (TR-FRET)
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: At least 6 months from date of receipt when stored as directed.

REFERENCES:

1. Lechner, M.G., *et al. Immunotherapy*. 2011 Nov; **3(11):** 1317–1340.

2. Ko, K., *et al., J. Exp. Med*. 2005; **202(7):** 885-891.

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ASSAY PROTOCOL:

All samples and controls should be tested in at least duplicate.

Protocol for GITR assay

1) Dilute one part **3x GITR TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x GITR Assay Buffer**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

2) Dilute **Anti-His Tb Donor** 100-fold in **1x GITR Assay Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

3) Dilute **Dye-labeled acceptor** 100-fold in **1x GITR Assay Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

4) Thaw **GITR, Biotinylated** on ice. Upon first thaw, briefly spin tube containing **GITR, Biotinylated** to recover the full contents of the tube. Aliquot into single-use aliquots. Store remaining undiluted **GITR** at −80°C immediately. *Note: **GITR, Biotinylated** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

5) Dilute **GITR, Biotinylated** in **1x GITR Assay Buffer** to 8 µg/ml. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

6) Prepare the master mixture: N wells x (3 µl diluted **GITR, Biotinylated** + 5 µl diluted **Anti-His Tb Donor** + 5 µl diluted **Dye-labeled acceptor**). Add 13 µl to every well.

6) Add 2 µl of inhibitor solution to each well designated “Test Inhibitor.” Add 2 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled “Negative Control” and “Positive Control.”

7) Add 5 µl **1x GITR Assay Buffer** to wells designated for “Negative Control.”

	Positive Control	**Negative Control**	**Test Inhibitor**
GITR, Biotinylated (diluted)	3 µl	3 µl	3 µl
Anti-His Tb Donor	5 µl	5 µl	5 µl
Dye-labeled Acceptor	5 µl	5 µl	5 µl
Test Inhibitor	–	–	2 µl
Inhibitor Buffer (no inhibitor)	2 µl	2 µl	–
1x GITR Assay Buffer	-	5 µl	-
GITRL-His (diluted)	5 µl	–	5 µl
Total	**20 µl**	**20 µl**	**20 µl**

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8) Thaw **GITRL-His** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **GITRL-His** into single-use aliquots. Store remaining undiluted **GITRL-His** in aliquots at −80°C immediately. *Note:* **GITRL-His** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

9) Dilute **GITRL-His** in **1x GITR Assay Buffer** to 0.2 µg/ml. Initiate reaction by adding 5 µl of diluted **GITRL-His** to wells designated for the "Positive Control" and "Test Inhibitor." Discard any remaining diluted **GITRL-His** protein after use.

10) Incubate at room temperature for 1.5 hours.

11) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	320±10 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	320±20 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 TR-FRET ratio (665 nm emission/620 nm emission).

If desired, data can be normalized to percent inhibition. Typically for inhibitor screens, the FRET value from the positive control is set to zero percent inhibition and the FRET value from the negative control is set to one hundred percent inhibition.

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EXAMPLE OF ASSAY RESULTS:

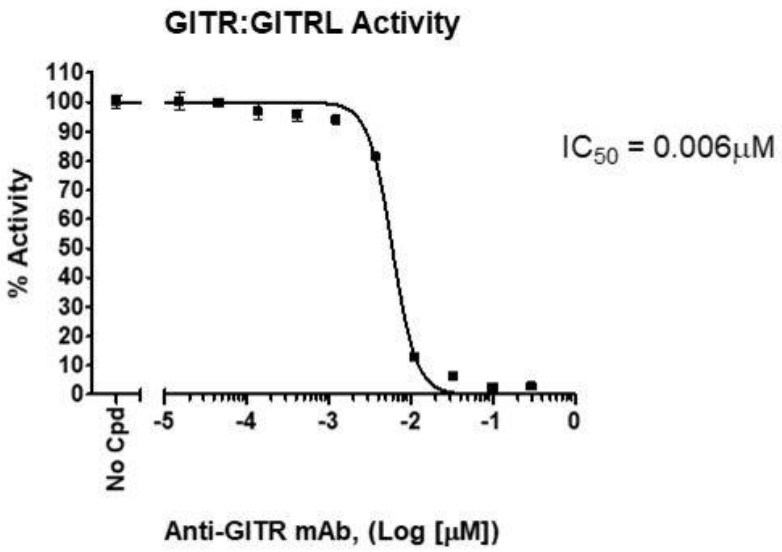

Figure Legend: Inhibition of GITR:GITRL interaction with anti-GITR antibody (BPS Bioscience, #79053). Data in the above graphs are expressed as FRET ratios. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Catalog #	Size
GITR - HEK293 Recombinant Cell Line	79092	2 vials
GITR / NF-κB Luciferase Reporter (Luc) - Jurkat Cell Line	60546	2 vials
GITRL CHO-K1 Recombinant Cell Line	60547	2 vials
GITRL:GITR[Biotinylated] Inhibitor Screening Assay Kit	72061	96 rxns.
Anti-GITR Antibody	79053	100 µg
Anti-GITR Antibody, PE-labeled	71295-1	50 µg
Anti-GITR Antibody, PE-labeled	71295-2	100 µg
GITRL, His-tag (Human)	71190	100 µg
GITR (CD357), Fc Fusion, Biotin-labeled (Human)	71256	50 µg
GITR (CD357), Fc fusion (Human)	71172	100 µg