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Data Sheet
CTLA4:B7-1 TR-FRET Assay
Catalog # 72120

DESCRIPTION:
The CTLA4:B7-1 TR-FRET Assay is designed to measure the inhibition of CTLA4 binding to B7-1 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 CTLA4, B7-1 protein, 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	
71152	CTLA4, biotinylated	15 µg	-80°C	***(Avoid freeze/thaw cycles!)***
71261	B7-1-Flag-Avi-His	5 µg	-80°C	
	Anti-His Tb Donor	2 x 10 µl	-20°C	
	Dye-labeled Acceptor	2 x 10 µl	-20°C	
	3x CTLA4 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 Resonance Energy 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.

REFERENCE: Huxley, P., *et al. Chem Biol.* 2004 Dec;**11(12):**1651-8.

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ASSAY PROTOCOL:

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

Protocol for CTLA4 assay

1) Dilute one part **3x CTLA4 TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x CTLA4 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 CTLA4 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 CTLA4 Assay Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

4) Thaw **CTLA4, Biotinylated** on ice. Upon first thaw, briefly spin tube containing **CTLA4, Biotinylated** to recover the full contents of the tube. Aliquot into single-use aliquots. Store remaining undiluted **CTLA4** at −80°C immediately. *Note: **CTLA4, Biotinylated** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

5) Dilute **CTLA4, Biotinylated** in **1x CTLA4 Assay Buffer** to 7.5 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 **CTLA4, 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 CTLA4 Assay Buffer** to wells designated for “Negative Control.”

	Positive Control	**Negative Control**	**Test Inhibitor**
CTLA4, 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 CTLA4 Assay Buffer	-	5 µl	-
B7-1-Flag-Avi-His (diluted)	5 µl	–	5 µl
Total	**20 µl**	**20 µl**	**20 µl**

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8) Thaw **B7-1-Flag-Avi-His** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **B7-1-Flag-Avi-His** into single-use aliquots. Store remaining undiluted **B7-1-Flag-Avi-His** in aliquots at −80°C immediately. *Note:* **B7-1-Flag-Avi-His** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

9) Dilute **B7-1-Flag-Avi-His** in **1x CTLA4 Assay Buffer** to 1.2 µg/ml. Initiate reaction by adding 5 µl of diluted **B7-1-Flag-Avi-His** to wells designated for the "Positive Control" and "Test Inhibitor." Discard any remaining diluted **B7-1-Flag-Avi-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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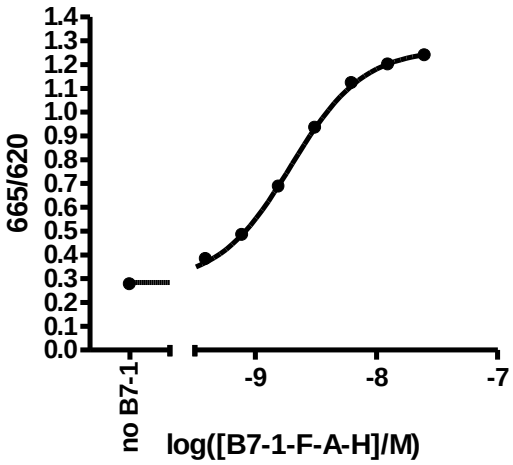
EXAMPLE OF ASSAY RESULTS:

A



B

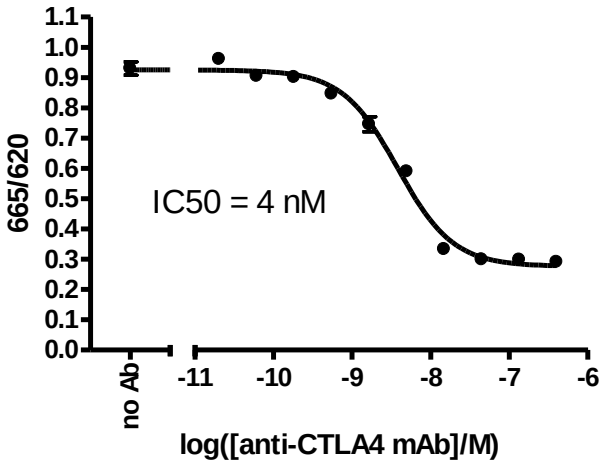


Figure Legend: Interaction (A) and Inhibition (B) of CTLA4 with B7-1. Data in the above graphs are expressed as FRET ratios. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Catalog #	Size
CTLA4 (CD152), Fc fusion	71149	100 µg
CTLA4 (CD152), Fc fusion, Biotin-labeled	71152	50 µg
B7-1 (CD80), FLAG-Avi-His-Tag	71261	100 µg
B7-1 (CD80), Fc fusion	71125	100 µg
B7-1 (CD80), Fc fusion, Biotin-labeled	71114	50 µg
B7-2 (CD86), FLAG-Avi-His-Tag, Biotin Labeled	71263	50 µg
B7-2 (CD86), Fc fusion	71150	100 µg
B7-2 (CD86), Fc fusion, Biotin-labeled	71149	50 µg
CTLA4[Biotinylated]:B7-1 Inhibitor Screening Kit	72009	96 rxns.
CTLA4[Biotinylated]:B7-2 Inhibitor Screening Kit	72024	96 rxns.
CD28:B7-1[Biotinylated] Inhibitor Screening Kit	72007	96 rxns.
CD28:B7-2[Biotinylated] Inhibitor Screening Kit	72062	96 rxns.
PD-L1:B7-1[Biotinylated] Inhibitor Screening Kit	72026	96 rxns.
Anti-CTLA4 Neutralizing Antibody	71212	100 µg
3x CLTA4 Assay Buffer	33297	100 ml