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Data Sheet

BRD2 (BD1+BD2) TR-FRET Assay Kit

Catalog # 32614
Size: 384 reactions

DESCRIPTION:
The BRD2 (BD1 + BD2) TR-FRET Assay Kit is designed to measure the inhibition of BRD2 (BD1 + BD2) binding to its substrate 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 terbium-labeled donor, dye-labeled acceptor, BRD2, substrate, and an inhibitor is incubated for sixty minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
31024	BRD2, BD1 and BD2 (65-459)	60 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand	50 µl	-80°C	
	Non-acetylated Ligand 1	15 µl	-20°C	
	Tb donor	20 µl	-20°C	
	Dye-labeled acceptor	20 µl	-20°C	
	3x BRDTR-FRET Assay Buffer	4 ml	-20°C	
Fisher 07-200-330	White, Nonbinding Corning, low volume, microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent 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(S): Filippakopoulos, P., *et al., Cell* 2012; **149**:214.

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

Protocol for BRD2 (BD1+BD2) assay

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

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

3) Add 5 µl of diluted **Tb-labeled donor**, and 5 µl of diluted **Dye-labeled acceptor** to each well designated "Test Inhibitor", "Ligand Control", and "Positive Control".

4) 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 "Ligand Control", and "Positive Control".

	Ligand Control	Positive Control	Test Inhibitor
Tb-labeled 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	2µl	–
BET Bromodomain Ligand		5 µl	5 µl
Non-acetylated Ligand 1	5 µl	–	–
BRD2 (BD1 + BD2) 50 ng/µl	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

5) Thaw **BET Bromodomain Ligand** and **Non-acetylated Ligand 1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot each ligand into single-use aliquots. Store remaining undiluted ligand at –80°C immediately. *Note: each ligand is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*

6) Individually dilute each ligand 40-fold in **1x BRD Assay Buffer**. Add 5 µL of diluted **BET Bromodomain Ligand** to each well designated as "Positive Control" and "Test Inhibitor". Add 5 µL of diluted **Non-acetylated Ligand 1** to the wells labeled as "Ligand Control".

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7) Thaw **BRD2** bromodomain protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **BRD2** protein into single-use aliquots. Store remaining undiluted **BRD2** in aliquots at –80°C immediately. *Note: BRD2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

8) Dilute **BRD2** in **1x BRD Assay Buffer** to 50 ng/µl (150 ng/reaction). Initiate reaction by adding 3 µl of diluted **BRD2** to wells designated for the "Ligand Control" "Positive Control", and "Test Inhibitor". Discard any remaining diluted BRD protein after use.

9) Incubate at room temperature for 1 hour.

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

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±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).

When percentage activity is calculated, the FRET value from the substrate (ligand) control can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\,Activity = \frac{FRET_S - FRET_{Sub}}{FRET_P - FRET_{Sub}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{Sub}$ = Substrate (ligand) control FRET, and $FRET_P$ = Positive control FRET.

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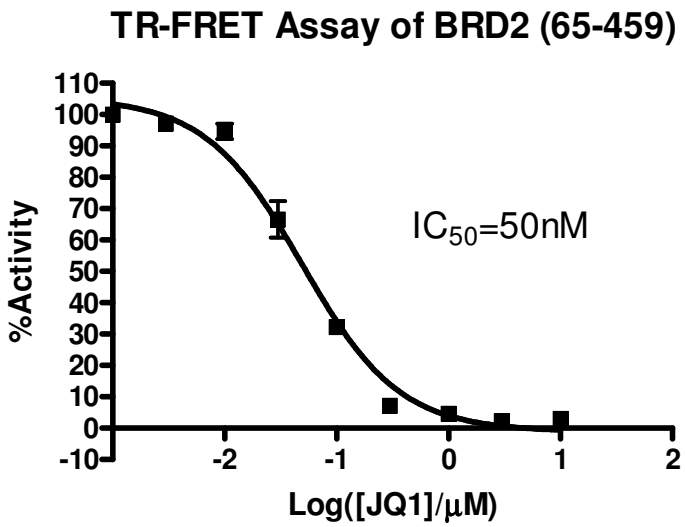
EXAMPLE OF ASSAY RESULTS:



Inhibition of BRD2 (BD1 + BD2) by (+)-JQ1, measured using the *BRD2 TR-FRET Assay Kit*, BPS Bioscience # 32614. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog	Size
BET Bromodomain Ligand	33000	0.5 mL
Bromodomain Non-acetylated Ligand 1	33005	0.5 mL
BRD1 (561 – 668), His-tag*	31010	100 µg
BRD2 (65 – 187), His-tag*	31022	100 µg
BRD2 (339 – 459), His-tag*	31020	100 µg
BRD2 (65 – 459), His-tag*	31025	100 µg
BRD3 (29 – 145), His-tag*	31030	100 µg
BRD3 (306 – 417), His-tag*	31031	100 µg
BRD4 (49 – 460), His-tag*	31045	100 µg
BRD4 (49 – 170), His-tag*	31042	100 µg
BRD4 (342 – 460), His-tag*	31043	100 µg
BRD1 Inhibitor Screening Kit	32521	384 rxns.
BRD2 (BD2) Inhibitor Screening Kit	32522	384 rxns.
BRD3 (BD1) Inhibitor Screening Kit	32513	384 rxns.
BRD3 (BD2) Inhibitor Screening Kit	32523	384 rxns.
BRD4 (BD1) Inhibitor Screening Kit	32514	384 rxns.
BRD4 (BD2) Inhibitor Screening Kit	32524	384 rxns.
(+)-JQ1 Inhibitor	27401	1 mg

*also available as GST-tag