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Data Sheet
BRD1 TR-FRET Assay Kit
Catalog # w42636

DESCRIPTION:
The BRD1 TR-FRET Assay Kit is designed to measure the inhibition of BRD1 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, BRD1, substrate, and an inhibitor is incubated for 120 minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
w41022	GST-BRD1	10 µg	-80℃	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand	50 µl	-80℃	
	Non-acetylated Ligand 1	15 µl	-80℃	
	Tb donor	2 x 10 µl	-20℃	
	Dye-labeled acceptor	2 x 10 µl	-20℃	
	3x BRD1 TR-FRET Assay Buffer	4 ml	-20℃	
	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.

1) Dilute one part **3x BRD1 TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x BRD1 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 BRD1 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 every well.

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 "Negative Control", and "Positive Control".

	Positive Control	Negative* 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	2 µl	–
BET Bromodomain Ligand	5 µl	–	5 µl
Non-acetylated Ligand 1	–	–	–
1x BRD1 Buffer	–	5 µl*	–
BRD1 (1 ng/µl)	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

***Non-acetylated Ligand 1** may be used as a substrate control in place of the negative control.

5) Thaw **BET Bromodomain Ligand** 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) Dilute **BET Bromodomain Ligand** 40-fold in **1x BRD1 Assay Buffer**. Add 5 µl of diluted **BET Bromodomain Ligand** to each well designated as "Positive Control" and "Test Inhibitor". Add 5 µl of **1x BRD1 Assay Buffer** to the wells labeled "Negative Control". " *Note: if using the **Non-acetylated Ligand 1**, dilute **Non-acetylated Ligand 1** 40-fold in **1x BRD1 Assay Buffer** and add 5 µl of diluted **Non-acetylated Ligand 1** to the "Negative Control" wells in place of the 5 µl of **1x BRD1 Assay Buffer** .*

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7) Thaw **GST-BRD1** bromodomain protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **GST-BRD1** protein into single-use aliquots. Store remaining undiluted **GST-BRD1** in aliquots at –80°C immediately. *Note: **GST-BRD1** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

8) Dilute **GST-BRD1** in **1x BRD1 Assay Buffer** to 1 ng/µl (3 ng/reaction). Initiate reaction by adding 3 µl of diluted **GST-BRD1** to every well. Discard any remaining diluted **GST-BRD1** protein after use.

9) Incubate at room temperature for 2 hours.

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 negative control can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

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

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

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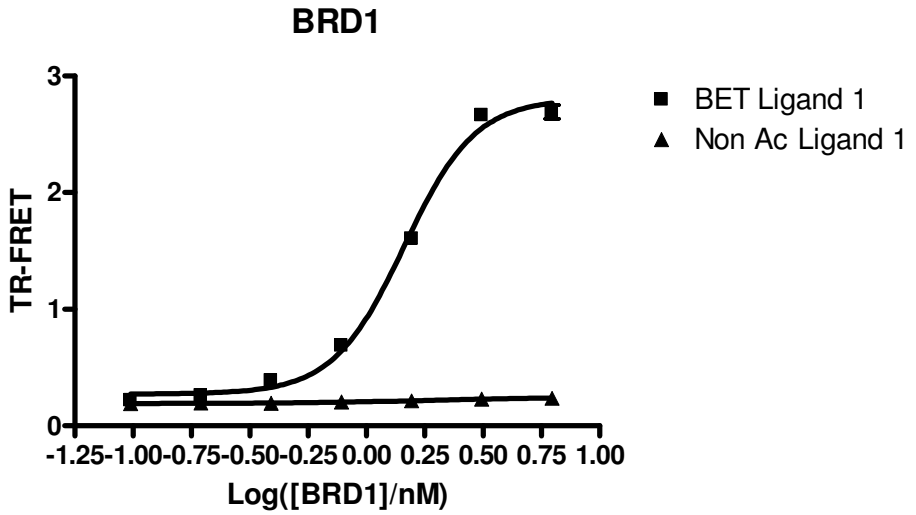
EXAMPLE OF ASSAY RESULTS:



Demonstration of BRD1 (West Bioscience #w41022) specificity in the presence of BET Bromodomain Ligand or Non-acetylated Ligand 1. Assay was done following protocol for WestBio Cat. #w42636. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com*

Note: Tb-labeled donor and dye-labeled acceptor are products of Cisbio Bioassays.