Data Sheet
BRD4 (BD1+BD2) TR-FRET Assay Kit
Catalog # AMS.32612
Size: 384 reactions

DESCRIPTION: The BRD4 (BD1+BD2) TR-FRET Assay Kit is designed to measure the inhibition of BRD4 (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, BRD4, substrate, and an inhibitor is incubated for 120 minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
31044	BRD4 (BD1+BD2)	10 µg	-80°C	***Avoid freeze/ thaw cycles!***
33000	BET Bromodomain Ligand	50 µl	-80°C	
33005	Non-acetylated Ligand 1	15 µl	-80°C	
	Tb-labeled donor	2 x 10 µl	-20°C	
	Dye-labeled acceptor	2 x 10 µl	-20°C	
33012	3x BRD TR-FRET Assay Buffer 1	4 ml	-20°C	
	White, Nonbinding, low volume, microtiter plate	1	Room temp.	

MATERIALS 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):
1. 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 BRD4 (BD1+BD2) Assay

1) Dilute one part **3x BRD TR-FRET Assay Buffer 1** with 2 parts distilled water (3-fold dilution) to make **1x BRD TR-FRET Assay Buffer 1**. 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 TR-FRET Assay Buffer 1**. 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," "Negative Control," and "Positive Control."

4) Add 2 µl of inhibitor solution to each well designated "Test Inhibitor." Add 2 µl of 10% DMSO in water (inhibitor buffer) to the wells labeled "Negative Control" and "Positive Control."

	Negative 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
10% DMSO in water (Inhibitor Buffer)	2 µl	2µl	–
BET Bromodomain Ligand		5 µl	5 µl
Non-acetylated Ligand 1	5 µl	–	–
BRD4 (BD1 + BD2) 6 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

Note: The BRD4 (BD1+BD2) TR-FRET Assay Kit is compatible with up to 0.5% final DMSO concentration.

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

7) Thaw **BRD4 (BD1 + BD2)** bromodomain protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **BRD4 (BD1 + BD2)** protein into single-use aliquots. Store remaining undiluted **BRD4** in aliquots at −80°C immediately. *Note: BRD4 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

8) Dilute **BRD4 (BD1 + BD2)** in **1x BRD TR-FRET Assay Buffer 1** to 6 ng/µl (18 ng/reaction). Initiate reaction by adding 3 µl of diluted **BRD4 (BD1 + BD2)** to wells designated for the "Negative Control," "Positive Control," and "Test Inhibitor." Discard any remaining diluted BRD 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).

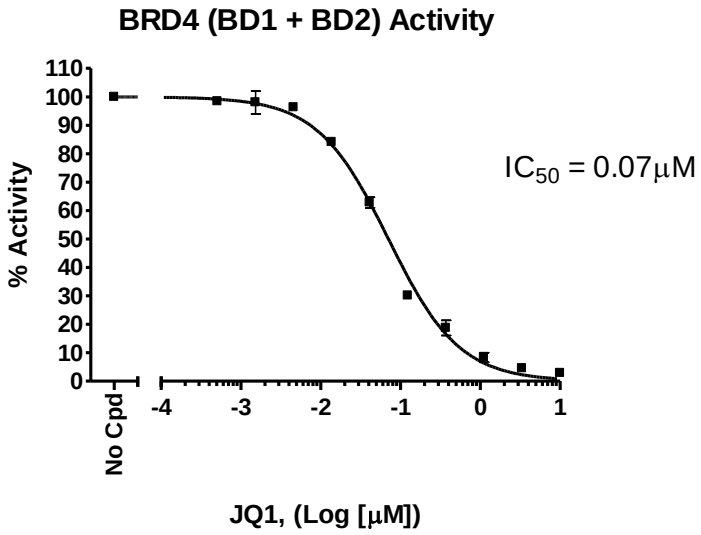
When percentage activity is calculated, the FRET value from the negative control can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

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

Where $FRET_s$ = Sample FRET, $FRET_{Neg}$ = Negative control FRET, and $FRET_P$ = Positive control FRET.

EXAMPLE OF ASSAY RESULTS:

TR-FRET Assay of BRD4 (BD1+BD2)
Inhibition by JQ1



Inhibition of BRD4 (BD1 + BD2) by JQ1 (AMS Cat. #27401), measured using the *BRD4 TR-FRETAssay Kit*, AMS # 32612. *Data shown is lot-specific.*

RELATED PRODUCTS:

Product	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 – 170), His-tag*	31042	100 µg
BRD4 (342 – 460), His-tag*	31043	100 µg
BRD4 (49 – 460), His-tag*	31045	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

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

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