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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet
JARID1B TR-FRET Assay Kit
Catalog #50514
Size: 384 reactions

DESCRIPTION: The *JARID1B TR-FRET Assay Kit* is designed to measure activity of JARID1B in a homogeneous 384 reaction format. JARID1B, also known as PLU-1 and KDM5B, is a JumonjiC (JmjC) and ARID domain-containing histone lysine demethylase that exhibits demethylation activity toward di- and trimethyl-lysine 4 (H3K4me2/3) on histone H3. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The *JARID1B TR-FRET Assay Kit* comes in a convenient format, with histone H3 peptide substrate, a Eu-labeled antibody against methylated K4 residue of Histone H3, demethylase assay buffer, TR-FRET detection buffer, dye-labeled acceptor, and purified JARID1B for 384 enzyme reactions. The key to the JARID1B Assay Kit is a highly specific antibody that recognizes demethylated substrate. With this kit, only three simple steps on a microtiter plate are required for demethylase activity detection. First, a sample containing JARID1B enzyme is incubated with the biotinylated substrate. Next, antibody is added. Finally, dye-labeled acceptor is added followed by fluorescence reading.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50121	JARID1B (KDM5A, RBBP2)	40 µg	-80°C	***Avoid freeze/ thaw cycles!***
	Eu-labeled K4 antibody	20 µl	-80°C	
	Biotinylated histone H3 peptide substrate 2	500 rxns	-80°C	
52407	4x HDM Assay Buffer 2	3 x 1 ml	-80°C	
52410	4x HDM Incomplete Buffer	2 x 1 ml	-20°C	
	Dye-labeled acceptor	2 x 10 µl	-20°C	
	TR-FRET Detection 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

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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.

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REFERENCE: Lahoud, M.H., *et al. Genome Res.* **11** (8): 1327–34.

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

Step 1:

1) Re-suspend lyophilized **Biotinylated histone H3 peptide substrate 2** in 500 µl of distilled water.

2) Prepare master mix: N wells × (2.5 µl **4x HDM Assay Buffer 2** + 1 µl **Biotinylated substrate** + 0.5 µl water).

3) Add 4 µl of master mixture to each well designated for the “Positive Control” and “Test Inhibitor”. For the "Blank", add 2.5 µl **4x HDM Incomplete Assay Buffer** + 1 µl **Biotinylated substrate** + 0.5 µl water. *Note: The **Incomplete buffer**, which does not contain α-ketoglutarate, provides a more accurate background value than a no-enzyme control.*

4) Thaw **JARID1B** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **JARID1B** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **JARID1B** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute **JARID1B** in **1x HDM Incomplete Assay Buffer** at 33.3 ng/µl (100 ng/3 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

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Reagent	Blank	Positive Control	Test Inhibitor
4x HDM Assay Buffer 2	–	2.5 µl	2.5 µl
4x HDM Incomplete Assay Buffer	2.5 µl	–	–
Biotinylated Substrate	1 µl	1 µl	1 µl
Distilled water	0.5 µl	0.5 µl	0.5 µl
Test Inhibitor	–	–	3 µl
Inhibitor buffer (no inhibitor)	3 µl	3 µl	–
JARID1B (33.3 ng/µl)	3 µl	3 µl	3 µl
Total	**10 µl**	**10 µl**	**10 µl**

6) Add 3 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 3 µl of the same solution without inhibitor (Inhibitor buffer)

7) Initiate reaction by adding 3 µl of diluted **JARID1B** prepared as described above. Incubate at room temperature for one hour. *Note: All incubations are done with slow shaking on a rotator platform.*

Step 2:

1) Thaw **TR-FRET Detection Buffer** and **Eu-labeled K4 antibody** on ice.

2) Dilute **Eu-labeled K4 antibody** 100-fold with **TR-FRET Detection Buffer**.

3) Add 5 µl per well. Incubate 30 minutes at room temperature with slow shaking.

Step 3:

1) Dilute **Dye-labeled acceptor** 100-fold with **TR-FRET Detection Buffer**.

2) Add 5 µl per well. Incubate for 1-2 hours at room temperature with slow shaking. *(Alternatively, dilute* **Eu-labeled K4 antibody** *(1:200) and* ***Dye-labeled acceptor*** *(1:200) with* ***TR-FRET Detection Buffer*** *in one step. Add 10 µL of Antibody/Acceptor mixture per well and incubate 1-2 hour.)*

3) Read the fluorescent intensity at 620 nm and 665 nm in a microtiter-plate reader capable of TR-FRET. See section below for details.

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Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	317±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	317±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:

Two sequential measurements should be conducted. Eu-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 (Blank or Substrate Control) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_p - FRET_{neg}} \times 100\%$$

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

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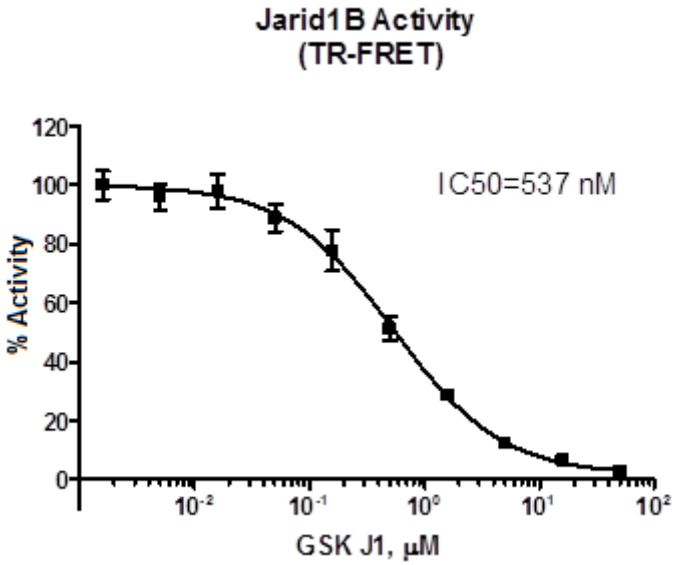
Example of Assay Results:



JARID1B enzyme activity, measured using the *JARID1B TR-FRET Assay Kit*, BPS Bioscience #50514. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS

Product Name	Catalog #	Size
JARID1B (KDM5B, PLU-1) enzyme	50121	20 µg
Mouse JARID1B enzyme	50122	20 µg
JARID1A (KDM5A) enzyme	50110	20 µg
JARID1C (KDM5A) enzyme	50112	20 µg
JARID1A (KDM5A) Chemiluminescent Kit	50513	96 rxns.
JARID1A (KDM5A) Homogeneous Kit	50510	384 rxns.
JARID1B (KDM5B) Homogeneous Kit	50512	384 rxns.
JARID1C (KDM5C) Homogeneous Kit	50511	384 rxns.
Anti–JARID1B polyclonal	25293	100 µl
Anti–JARID1C polyclonal	25294	100 µl
Anti–JARID2 polyclonal	25295	100 µl