6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

JARID1A Chemiluminescent Assay Kit

Catalog # 50513

DESCRIPTION: The *JARID1A Chemiluminescent Assay Kit* is designed to measure JARID1A activity for screening and profiling applications. JARID1A is a member of the Jumonji, AT-rich interactive domain 1 (JARID1) histone demethylase protein family. It binds directly, with several other proteins, to retinoblastoma protein which regulates cell proliferation. The *JARID1A Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well strip plate precoated with methylated histone H3 peptide substrate, primary antibody, the secondary HRP-labeled antibody, demethylase assay buffer, and purified JARID1A for 100 enzyme reactions. The key to the *JARID1A Chemiluminescent 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 detection of demethylase activity. First, JARID1A enzyme is incubated with the methylated H3 peptide for one hour. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by the addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50110	JARID1A (KDM5A, RBBP2)	10 µg	-80°C	***Avoid freeze/ thaw cycles!***
52140M	Primary antibody 13	25 µl	-80°C	
52130H	Secondary HRP-labeled antibody 1	10 µl	-80°C	
	4x JARID1A Direct Assay Buffer	3 x 1 ml	-80°C	
52100	Blocking buffer 4	50 ml	+4°C	
79670	ELISA ECL chemiluminescent substrate A (transparent bottle)	6 ml	+4°C	
79670	ELISA ECL chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	8-well strip plate module precoated with histone substrate	1	+4°C	

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

"*Note: *The buffer in this kit was reformulated in May of 2015 with a different reducing agent to improve assay performance. The old formulation can still be purchased upon special request."*

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

STABILITY: One year from date of receipt when stored as directed.

REFERENCE: Klose, R.J., *et al. Cell* 2007; **128:** 889.

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

Step 1:

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 5 minutes at room temperature. Tap the strip plate onto clean paper towels to remove liquid.

2) Dilute **4X JARID1A Direct Assay Buffer** with water to make **1x JARID1A Direct Assay Buffer**. Add 20 µl **1x JARID1A Direct Assay Buffer** to each well.

3) Thaw **JARID1A** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **JARID1A** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: JARID1A is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **JARID1A** in **1X JARID1A Direct Assay Buffer** at 1 ng/µl (25 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) Add 5 µl of inhibitor solution of each well designated "Test Inhibitor." For the "Positive Control" and "Blank," add 5 µl of 5% DMSO in water (Inhibitor buffer). *Note: Keep final DMSO concentration ≤1%.*

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

	Positive Control	Test Inhibitor	Blank
1X JARID1A Direct Assay Buffer	20 µl	20 µl	45 µl
Test Inhibitor	–	5 µl	–
5% DMSO in water (Inhibitor buffer)	5 µl	–	5 µl
JARID1A (1 ng/µl)	25 µl	25 µl	–
Total	**50 µl**	**50 µl**	**50 µl**

6) Add 25 µl of **1x JARID1A Direct Assay Buffer** to wells designated as "Blank." Initiate reaction by adding 25 µl of **diluted JARID1A** prepared as described above to wells designated "Positive Control" and "Test Inhibitor." Incubate at room temperature for one hour.

7) Wash the strip plate three times with TBST buffer. Blot dry onto clean paper towels.

Step 2:

1) Dilute **Primary antibody 13** 400-fold with **Blocking Buffer 4**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash strip plate three times with TBST buffer.

Step 3:

1) Dilute **Secondary HRP-labeled antibody 1** 1,000-fold with **Blocking Buffer 4**.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash strip plate three times with TBST buffer.

4) Just before use, mix on ice 50 µl **ELISA ECL chemiluminescent substrate A** and 50 µl **ELISA ECL chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

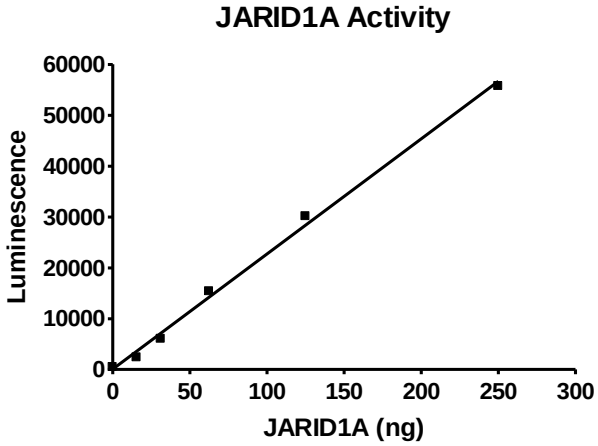
5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

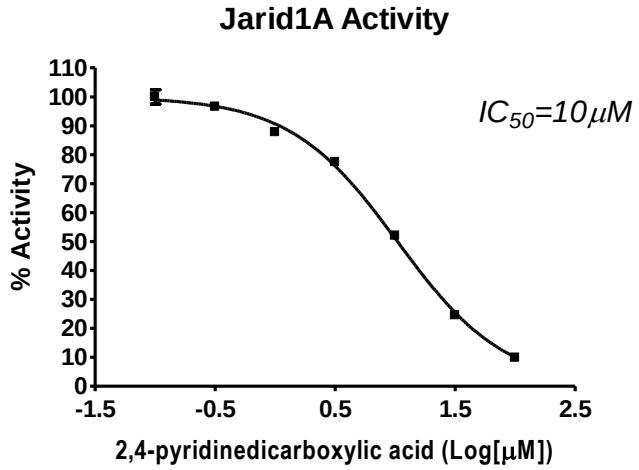
To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Examples of Assay Results:



JARID1A enzyme activity, measured using the *JARID1A Chemiluminescent Assay Kit*, BPS Bioscience #50513. *Data shown is lot-specific*

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com



JARID1A enzyme inhibition by 2-4-pyridinedicarboxylic acid, measured using the *JARID1A Chemiluminescent Assay Kit*, BPS Bioscience #50513. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS

Product Name	Catalog #	Size
JARID1A recombinant protein	50110	20 µg
JARID1A recombinant protein, His/Avi-tag	50155	20 µg
JARID1B recombinant protein	50121	20 µg
JARID1C recombinant protein	50112	20 µg
JMJD2C recombinant protein	50105	100 µg
LSD1 recombinant protein	50100	50 µg
JARID1A Homogeneous Assay Kit	50510	384 reactions
JARID1B Homogeneous Assay Kit	50512	384 reactions
JARID1C Homogeneous Assay Kit	50511	384 reactions
JMJD2A Homogeneous Assay Kit	50413	384 reactions
JMJD2B Homogeneous Assay Kit	50414	384 reactions
JMJD2C Homogeneous Assay Kit	50415	384 reactions
JMJD2C Chemiluminescent Assay Kit	50405	96 reactions
JMJD2D Chemiluminescent Assay Kit	50418	96 reactions
JMJD3 Chemiluminescent Assay Kit	50406	96 reactions
LSD1 Chemiluminescent Assay Kit	50109	96 reactions

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.
To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**
Or you can Email us at: **info@bpsbioscience.com**
Please visit our website at: **www.bpsbioscience.com**

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is same as "blank" value.	JARID1A has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh JARID1A, BPS Bioscience #50110. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection. See section on "Reading Chemiluminescence" above.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Be sure to include blocking steps after wash steps. Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of JARID1A, BPS Bioscience #50110 to create a standard curve.