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Data Sheet

ALKBH5 Chemiluminescent Assay Kit

Catalog #79659

BACKGROUND: ALKBH5 (AlkB Homolog 5, RNA Demethylase) is an enzyme that removes N6-Methyladenosine (m^6A) from target mRNAs. ALKBH5 has been connected to various cancers, viral replication, and metabolic diseases while also being shown to remove the alkylation damage and restore adenine in DNA.

DESCRIPTION: The *ALKBH5 Chemiluminescent Assay Kit* is designed to measure ALKBH5 activity for screening and profiling applications. The *ALKBH5 Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well strip plate precoated with ALKBH5 substrate, primary antibody, HRP-labeled secondary antibody, demethylase assay buffer, and purified ALKBH5 for 96 enzyme reactions. The key to the *ALKBH5 Chemiluminescent Assay Kit* is a highly specific antibody that recognizes methylated substrate. Signal is inversely related to ALKBH5 demethylase activity. With this kit, only three simple steps on a microtiter plate are required for detection of demethylase activity. First, ALKBH5 enzyme is incubated with the substrate. 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	
100057	ALKBH5	100 µg	-80°C	***Avoid freeze/ thaw cycles!***
52140Z4	Primary antibody 29	100 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
	4x ALKBH5 assay buffer	3 x 1 ml	-80°C	
79556	Blocking buffer 1	50 ml	+4°C	
79670	ELISA ECL substrate A (transparent bottle)	6 ml	Room Temp	
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temp	
	White 8-well strip plate module precoated with RNA substrate*	1	+4°C	

*** NOTE: It is critical to perform all steps of the assay using RNAse-free conditions.**

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MATERIALS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20), prepared with DEPC-treated water.
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: 6 months from date of receipt, when stored as directed.

REFERENCE: Zheng, G., Dahl, J.A., *et al.* 2013. ALKBH5 Is a Mammalian RNA Demethylase that Impacts RNA Metabolism and Mouse Fertility, *Molecular Cell*, 49(1):18-29.

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

Step 1:

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the strip plate onto clean paper towels to remove liquid.

	Blank	Positive Control	Test Inhibitor
4x ALKBH5 assay buffer	7.5 µl	7.5 µl	7.5 µl
RNAse-free distilled water	17.5 µl	17.5 µl	17.5 µl
Test Inhibitor/Activator	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	–
1x ALKBH5 buffer	20 µl	–	–
Diluted ALKBH5 (50 ng/µl)	–	20 µl	20 µl
Total	50 µl	50 µl	50 µl

2) Prepare master mix: N wells × (7.5 µl **4x ALKBH5 Assay Buffer** + 17.5 µl RNAse-free water). Add 25 µl of master mixture to each well.

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3) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor.” For the “Positive Control" and "Blank," add 5 µl of the same solution without inhibitor (Inhibitor buffer). *Important: Keep final DMSO concentration ≤1%.*

4) Prepare **1x ALKBH5 Buffer** by diluting 1 part **4x ALKBH5 Assay Buffer** with three parts distilled water. Prepare only enough **1x ALKBH5 Buffer** for the assay. Add 20 µl of **1x ALKBH5 buffer** to wells designated as "Blank."

5) Thaw **ALKBH5** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **ALKBH5** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: ALKBH5 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute **ALKBH5** in **1x ALKBH5 Buffer** at 50 ng/µl (1000 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

7) Initiate reaction by adding 20 µl of **diluted ALKBH5** prepared as described above to wells designated "Positive Control" and "Test Inhibitor." Cover the plate and incubate overnight at room temperature with slow shaking. Glue the wells if necessary.

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

9) Add 100 µl of **Blocking buffer 1** to every well. Shake on a rotating platform for 10 minutes. Remove supernatant as described above.

Step 2:

1) Dilute **Primary antibody 29** 100-fold with **Blocking Buffer 1**.

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

3) Remove supernatant from the wells and wash the strip three times with 200 µl of TBST buffer and incubate in **Blocking Buffer 1** as described in steps 1-8 and 1-9.

Step 3:

1) Dilute **Secondary HRP-labeled antibody 2** 1,000-fold with **Blocking Buffer 1**.

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

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3) Remove supernatant from the wells and wash the strip three times with 200 µl of TBST buffer and incubate in **Blocking Buffer 1** as described in steps 1-8 and 1-9.

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

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 40000). Make sure signal decrease corresponds to increased activity.

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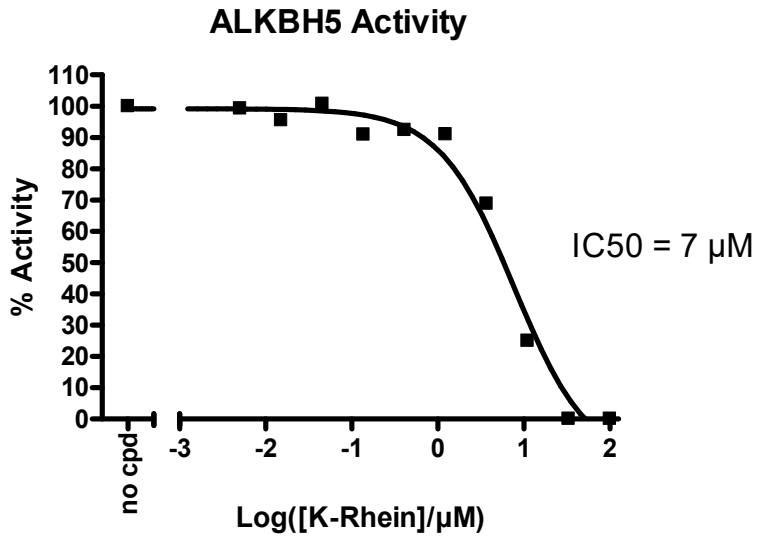
Examples of Assay Results:



ALKBH5 enzyme inhibition by K-Rhein measured using the *ALKBH5 Chemiluminescent Assay Kit*, BPS Bioscience #79659. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS

Product Name	Catalog #	Size
ALKBH5, FLAG-Tag	100057	20 µg
FTO(ALKBH9), His-Tag	79480	20 µg
Adenosine Deaminase (ADA), His-tag	70016	100 µg
MTAP, GST-tag	50305	50 µg
FTO Chemiluminescent Assay Kit	79344	96 rxns.
JHDM1D (KDM7A) recombinant protein	50419	20 µg
JMJD1A (KDM3A) recombinant protein	50130	20 µg
JARID1A recombinant protein	50155	20 µg
JMJD2A recombinant protein	50123	100 µg
LSD1 recombinant protein	50100	50 µg