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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The KAT8 (MOF) Chemiluminescence Assay Kit is an ELISA-based assay designed to measure the histone acetyl transferase activity of KAT8 (lysine acetyltransferase 8, also known as MOF) for screening and profiling applications. This kit comes in a convenient 96-well format with enough purified KAT8, acetyl donor, histone substrate, all the reagents necessary for assay detection, and blocking buffer for 96 enzyme reactions.

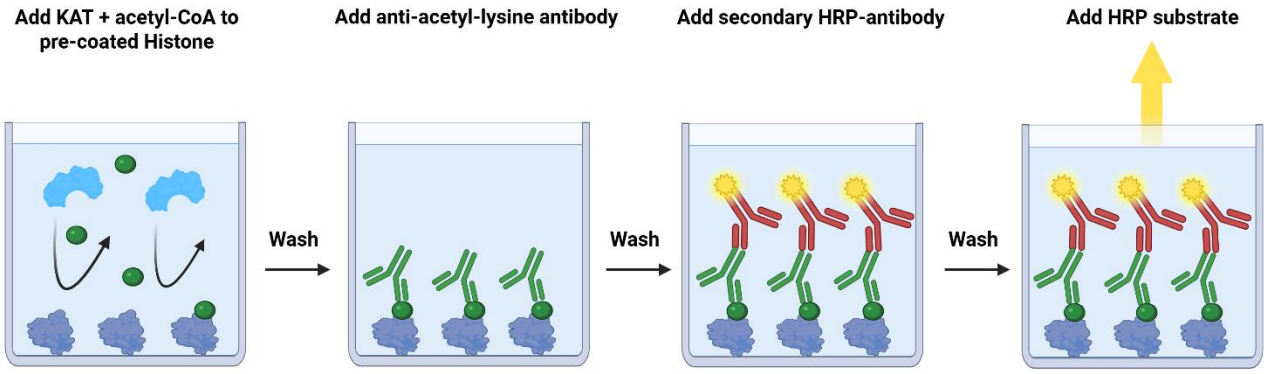

Figure 1. KAT8 (MOF) Chemiluminescence Assay Kit schematic.

KAT8 protein is added to a plate with a histone substrate in the presence of acetyl-CoA. After washing, an anti-acetyl-lysine antibody is added, followed by a secondary HRP-antibody. Lastly, HRP substrate is added and the chemiluminescence signal generated can be measured. The signal is proportional to the acetyltransferase activity of KAT8.

Background

KAT8 (lysine acetyltransferase 8), also known as MOF, is a histone acetyltransferase which adds acetyl groups to histone H4 at lysine 16. KAT8 activity impacts chromatin regulation, DNA damage repair, cell differentiation, and apoptosis. KAT8 dysfunction can result in development and metastasis of AML (acute myeloid leukemia) and other cancer types. Small molecule KAT8 inhibitors have been developed in efforts to further the study of KAT8 function and its impact in cancer progression. KAT8 has also been identified as a risk gene for the development of PD (Parkinson's disease), due to its role in Parkin-dependent mitophagy. Few inhibitors have been described and most present lot selectivity. Further studies will elucidate the exact role of this protein and how best to target it for therapeutic purposes.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
52028	Histone H4, Full Length, His-Tag*	10 µg	-80°C
102167	KAT8 (MOF), FLAG-Tag*	10 µg	-80°C
82967	AT-02 Buffer	20 ml	-80°C
52100	Blocking Buffer 4	50 ml	-80°C
82966	Primary Antibody 34	5 µl	-80°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-80°C
82735	0.5M DTT	200 µl	-80°C
82946	1 mM Acetyl-CoA	50 µl	
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- TBS: TRIS-buffered saline pH 7.4
- TBST Buffer (1x TBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This assay kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive Control”, and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using Anacardic Acid (MedChem#HY-N2020) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Coat 96-well plate
Coat the plate one day prior to running your samples.

1. Thaw **Histone H4** on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute Histone H4 to 2 ng/µl with 1x TBS (50 µl/well).
3. Add 50 µl of diluted Histone H4 to every well.
4. Incubate at 4°C overnight.
5. Wash the plate three times using 200 µl of TBST Buffer per well.
6. Tap the plate onto a clean paper towel to remove the liquid.
7. Block the wells by adding 200 µl of **Blocking Buffer 4** to every well.
8. Incubate at Room Temperature (RT) for at least 60 minutes.
9. Wash the plate three times using 200 µl of TBST Buffer per well.
10. Tap the plate onto a clean paper towel to remove the liquid.

Step 2: Reaction

1. Prepare **Assay Buffer** by diluting **0.5 M DTT** 500-fold with **AT-02 Buffer**.
2. Thaw **KAT8** on ice. Briefly spin the tube containing the protein to recover its full content.
3. Dilute KAT8 to 5 ng/µl with Assay Buffer (20 µl/well).
4. Add 20 µl **of diluted KAT8 the** "Positive Control" and "Test Inhibitor wells" wells.
5. Add 20 µl of Assay Buffer to the "Blank" wells.
6. Prepare the **Test Inhibitor** (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

3.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using Assay Buffer.

For positive and negative controls, use Assay Buffer (Diluent Solution).

OR

3.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

7. Add 5 µl of diluted Test Inhibitor to each well labeled as "Test Inhibitor".

8. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.

9. Prepare the **Acetyl-CoA Substrate Solution** (25 µl/well) by diluting **1 mM Acetyl-CoA** 50-fold with Assay Buffer.

10. Add 25 µl of **Acetyl-CoA Substrate Solution** to all wells.

11. Incubate at RT for 4 hours.

	Blank	**Positive Control**	**Test Inhibitor**
Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted KAT8 (5 ng/µl)	-	20 µl	20 µl
Acetyl-CoA substrate solution	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 200 µl of TBST Buffer per well and tap the plate onto a clean paper towel.

Step 3: Detection

1. Dilute 1000-fold the **Primary Antibody 34** with **Blocking Buffer 4** (50 µl/well).
2. Add 50 µl of diluted Primary Antibody 34 to every well.
3. Incubate for 1 hour at RT.
4. Wash the plate three times with 200 µl of TBST Buffer per well and tap the plate onto a clean paper towel.
5. Dilute 1000-fold the **Secondary Antibody 2** with Blocking Buffer 4 (50 µl/well).
6. Add 50 µl of diluted Secondary Antibody 2 to every well.
7. Incubate at RT for 45 minutes.
8. Wash the plate 3 times using 200 µl of TBST per well.
9. Just before use, mix 1 volume of **ELISA ECL Substrate A** and 1 volume of **ELISA ECL Substrate B** (100 µl of mix/ well).
10. Add 100 µl of mix to every well.
11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
12. The "Blank" value should be subtracted from all other values.

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 controls.

Example Results

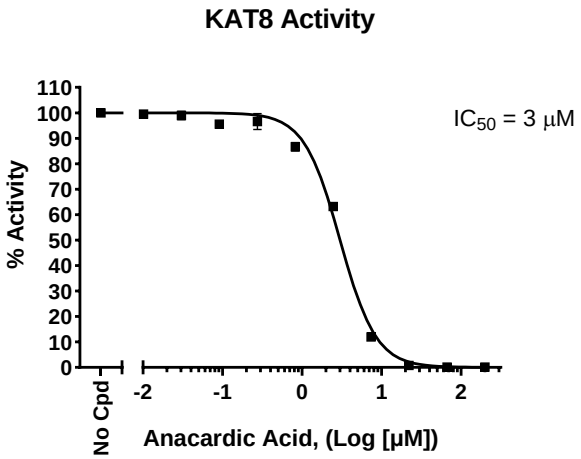

Figure 2: Inhibition of KAT8 by Anacardic Acid.
KAT8 was incubated with increasing concentrations of Anacardic Acid (MedChem#HY-N2020) in a Histone H4 coated plate. Luminescence was measured using a Bio-Tek microplate reader.

Data shown is representative.

References

Fiorentino F., *et al.*, 2023 *Journal of Medicinal Chemistry* 66 (10): 6591-6616.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
GCN5 Chemiluminescence Assay Kit	50079L	96 reactions
P300 Chemiluminescence Assay Kit	79705	96 reactions
KAT6B (MORF) Chemiluminescence Assay Kit	83536	96 reactions
KAT1 (HAT1) Chemiluminescence Assay Kit	82959	96 reactions

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