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Produktinformation

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

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The KAT6B (MORF) Chemiluminescent Assay Kit is an ELISA-based assay designed to measure the histone acetyl transferase activity of KAT6B (lysine acetyltransferase 6B, also known as MORF) for screening and profiling applications. This kit comes in a convenient 96-well format with enough purified KAT6B (amino acids 710-1020), acetyl donor, pre-coated plate with histone substrate, all the reagents necessary for assay detection, and blocking buffer for 96 enzyme reactions.

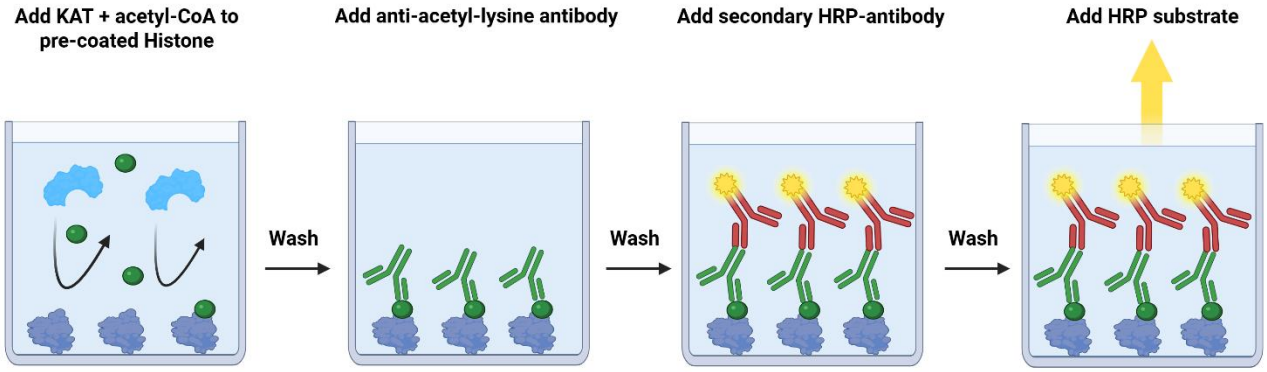

Figure 1. KAT6B (MORF) Chemiluminescent Assay Kit schematic.
KAT6B protein is added to a plate pre-coated with a histone substrate in the presence of acetyl-CoA. After washing, an anti-acetyl-lysine antibody is added, followed by a secondary HRP-antibody. Lastly, HRP substrate is added and the chemiluminescence signal generated can be measured. The signal is proportional to the acetyltransferase activity of KAT6B.

Background

KAT6B, or Lysine Acetyltransferase 6B, also known as MORF, is a histone acetyltransferase of the MYST family and plays a crucial role in chromatin organization and gene expression regulation by acetylating histones, which impacts DNA accessibility and transcription. It is part of the MOZ/MORF protein complex. KAT6B is involved in various biological processes, including brain development and RUNX2 (Runt-related transcription factor 2)-dependent transcriptional activation 1. Mutations in the KAT6B gene are associated with several rare genetic disorders, primarily affecting neurodevelopment. It is also linked to cancer, with its role as tumor suppressor or oncogene being type dependent. Inhibitors of KAT6A and KAT6B have been developed to block acetylation activity, showing promising clinical trial results in slowing tumor progression of breast cancer patients.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
102175	KAT6B (MORF), FLAG-Tag*	10 µg	-80°C
52028	Histone H4, Full Length, His-Tag*	10 µg	-80°C
82946	1mM Acetyl-CoA	5 µl	-20°C
79708	2x HAT Assay Buffer	10 ml	-20°C
52100	Blocking Buffer 4	50 ml	+4°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	-20°C
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 plate		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 WM-1119 (MedChem#HY-102058) 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: Coating
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 **1x Assay Buffer** (1x HAT Assay Buffer with 1 mM DTT) by diluting **0.5 M DTT** 250-fold with **2x HAT Assay Buffer** following by a 2-fold dilution with distilled water.
2. 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.

   2.1 If the Test Inhibitor/Blocker is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

2.2 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 1x 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 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x 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%.

3. Thaw **KAT6B** on ice. Briefly spin the tube containing the enzyme to recover its full content.
4. Dilute KAT6B to 5 ng/µl with 1x Assay Buffer (20 µl/well).
5. Add 20 µl of diluted KAT6B to the "Positive Control" and "Test Inhibitor" wells.
6. Add 20 µl of 1x Assay Buffer to the "Blank" wells.
7. Add 5 µl of diluted inhibitor to the "Test Inhibitor" wells.
8. Add 5 µl of Diluent Solution to the "Blank" and "Positive Control" wells.
9. Prepare the **Acetyl-CoA Substrate** Solution (25 µl/well) by diluting **1 mM Acetyl-CoA** 500-fold in 1x Assay Buffer.
10. Add 25 µl of Acetyl-CoA Substrate Solution to all wells.
11. Incubate plate at RT for 4 hours.

	Blank	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted KAT6B (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 3 times using 200 µl of TBST per well.
13. Tap the plate onto clean paper towel to remove the liquid.

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 at RT for 1 hour.
4. Wash the plate 3 times using 200 µl of TBST per well.
5. Tap the plate onto a clean paper towel to remove the liquid.
6. Dilute 1000-fold the **Secondary Antibody 2** with Blocking Buffer 4 (50 µl/well).
7. Add 50 µl of diluted Secondary Antibody 2 to every well.
8. Incubate at RT for 45 minutes.
9. Wash the plate 3 times using 200 µl of TBST per well.
10. Tap the plate onto a clean paper towel to remove the liquid.
11. Just before use, mix 1 volume of **ELISA ECL Substrate A** and 1 volume of **ELISA ECL Substrate B** (100 µl of mix/ well).
12. Add 100 µl of mix to every well.
13. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
14. 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.

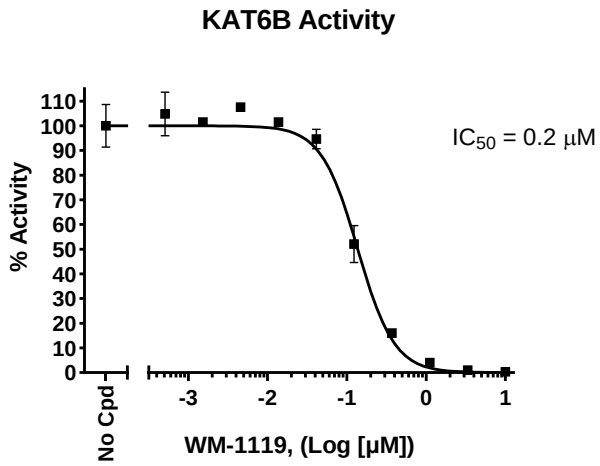

Figure 2: Inhibition of KAT6B by WM-1119.
KAT6B was incubated with increasing concentrations of WM-1119 (MedChem#HY-102058) in a pre-coated plate. Luminescence was measured using a Bio-Tek microplate reader.

Data shown is representative.

References

Jeselsohn R., and Polyak K., *et al.*, 2023 *Cell Chemical Biology* 30 (10): P1183-1185.
Wiesel-Motiuk N. and Assaraf Y., 2020 *Drug Resistance Updates* 53:100729.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions.

Related Products

Products	*Catalog #*	*Size*
GCN5 Chemiluminescence Assay Kit	50079L	96 reactions
P300 Chemiluminescence Assay Kit	79705	96 reactions
KAT1 (HAT1) Chemiluminescence Assay Kit	82959	96 reactions
KAT8 (MOF) Chemiluminescence Assay Kit	83537	96 reactions

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