

KAT7 (K277R), Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog # **K316-382BG**

Lot # K4573-4

Product Description

Recombinant full-length human KAT7 (K277R) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_007067](#).

Alternative Name(s)

HBO1; HBOA; MYST2; ZC2HC7

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

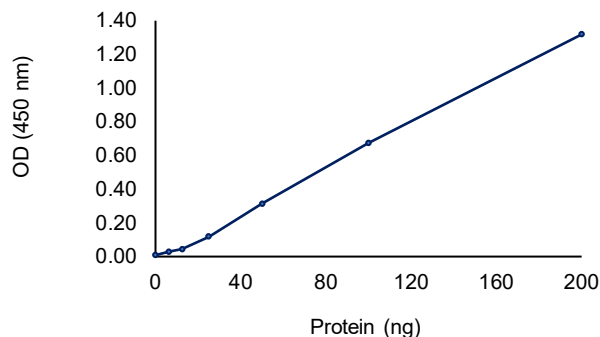
Scientific Background

KAT7 or K (lysine) acetyltransferase 5 is a signaling protein that belongs to the MYST family of histone acetyltransferases (HATs) and was originally isolated as an HIV-1 TAT-interactive protein which play important roles in regulating chromatin remodeling, transcription and other nuclear processes by acetylating histone and nonhistone proteins. KAT7 acts as a transcriptional inhibitor and inhibited AR-mediated transactivation of reporter constructs in CV-1 and PC-3 cells (1). KAT7 is part of a multisubunit complex that can acetylate histones H3 and H4 (2).

References

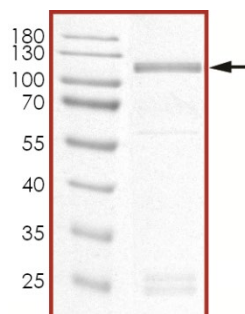
- Sharma, M. et.al: Androgen receptor interacts with a novel MYST protein, HBO1. J. Biol. Chem. 275: 35200-35208, 2000.
- Iizuka, M. et.al: Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. J. Biol. Chem. 274: 23027-23034, 1999.

Specific Activity



The specific activity of KAT7 (K277R) was determined to be **785 ng/min/mg** as per activity assay protocol.

Purity



The purity of KAT7 (K277R) was determined to be **>90%** by densitometry.
Observed MW **~110 kDa**.
Calculated MW **~95 kDa**.

KAT7 (K277R), Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	K316-382BG
Specific Activity	785 ng/min/mg
Lot #	K4573-4
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Acetyltransferases (Catalog #: K316-382BG)

Active KAT7 (K277R) (0.1 µg/µl) diluted with Acetyltransferase Dilution Buffer (Catalog #: A21-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active KAT7 (K277R) for optimal results).

Acetyltransferase Dilution Buffer (Cat. #: A21-09)

Acetyltransferase Dilution Buffer diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution prior to use.

Acetyltransferase Assay Buffer (Cat. #: A01-09)

Buffer components: 250mM Tris-HCl, pH 8.0, 0.5mM EDTA, 25% glycerol. Add 2mM DTT to Acetyltransferase Assay Buffer prior to use.

Acetyl-CoA Solution

Acetyl Co-enzyme A sodium salt (Sigma, Cat. # A2056), was diluted in 10mM sodium acetate, pH 5.0 to make a stock solution at a final concentration of 500 µM.

Substrate (Catalog#: H12-58)

Histone H3 Peptide (1-21) diluted in distilled H₂O to a final concentration of 1 mg/ml.

Standard

Acetylated Histone H3 peptide (1-21) (Catalog #: H12-358) may be used as a standard.

Acetyl Lysine Detection Antibody, rabbit polyclonal, biotinylated

The biotinylated anti-acetyl lysine antibody was from Immunechem (Catalog#: ICP 0383). The streptavidin HRP was from R@D Systems (Catalog#: DY998). TMB substrate was from Biopanda (Catalog#: TMB-S-004)

Assay Protocol

- Step 1.** Dilute Histone H3 Peptide (1-21) (**Substrate**) in 10mM Na₂CO₃ to 0.4 µg/ml. Dilute Acetylated Histone H3 conjugate to 2µg/ml (**Standard**), then make 2-fold dilution for 8 dilutions (standard). Add 50µl/well of either the Histone H3 Peptide or Standard to 94 wells of a 96 well ELISA plate, leaving 2 wells without coating as **Blank** control. Incubate in 4°C overnight.
- Step 2.** Discard solution. Wash plate 3 times with ELISA Wash buffer (1X PBS with 0.05% Tween-20).
- Step 3.** Block plate with 200µl/well of 1% BSA in PBS. Incubate at room temperature for 2 hours.
- Step 4.** Wash the plate by repeating Step 2.
- Step 5.** Thaw the Active KAT7 (K277R) enzyme, Acetyltransferase Assay Buffer, and Acetyltransferase Dilution Buffer on ice.
- Step 6.** Add the following reaction components to substrate coated wells, bringing the initial reaction volume up to 40µl.
 - Component 1.** 10µl of diluted Active KAT7 (K277R) (Catalog #: K316-382BG)
 - Component 2.** 30µl of Acetyltransferase Assay Buffer (Catalog #: A01-09)
- Step 7.** Add 40µl of Acetyltransferase Dilution Buffer to **Standard** wells and **Blank** control wells.
- Step 8.** Initiate the reaction by the addition 10µl of 500 µM Acetyl -CoA solution bringing the final volume of all wells up to 50µl, and a final concentration of Acetyl -CoA of 100 µM. Incubate the mixture at 37°C for 1 hour.
- Step 9.** Wash the plate by repeating step 2.
- Step 10.** Dilute Biotinylated anti-acetyl lysine antibody to 0.125 ug/ml in PBS with 1% BSA. Add 50ul/well and incubate at room temperature for one hour.
- Step 11.** Wash the plate by repeating step 2
- Step 12.** Dilute streptavidin HRP in PBS with 1% BSA. Add 50µl/well and incubate at room temperature with slow shaking for 30 minutes.
- Step 13.** Discard solution and wash plate 6 times with ELISA Wash buffer.
- Step 14.** Add 50µl/well of TMB Substrate and incubate approximately 10-15 minutes for blue color development.
- Step 15.** Stop the reaction by adding 50µl/well of 2N sulfuric acid, turning the blue color to yellow.
- Step 16.** Read the absorbance in TECAN microplate reader at 450 nm and 570 nm.
- Step 17.** Calculate average duplicate readings for sample wells, standard wells and blank wells, if required.
- Step 18.** Generate a standard curve as OD value versus amount of standard at each concentration point. Then calculate the corresponding ng of acetylated product from the standard curve.
- Step 19.** Calculate the KAT specific activity as outlined below.

KAT Specific Activity (SA) (ng/min/mg)

KAT Activity (ng/min/mg) = Acetylated product (ng) / (Reaction time in min) * (Enzyme amount in mg)

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SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: KAT7 (K277R), Active

Catalog # K316-382BG

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: SignalChem Biotech Inc.
 Street Address: 110-13120 Vanier Place
 City, Prov. Postal Code: Richmond, BC, V6V 2J2
 Fax: 604-232-4601
 EMERGENCY PHONE: 604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixtures.

Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	≤25%
NaCl	Sodium chloride	7647-14-5	≤1.753 %
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 - Amino - 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	1185-53-1	<0.8%
Glutathione	Glutathione	70-18-8	0.307%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
EDTA	Ethylenediaminetetracetic acid	6381-92-6	0.0037%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m ³ for mist	British Columbia, Canada
TWA	3 mg/m ³ for respirable mist	British Columbia, Canada
TWA	10 mg/m ³	Alberta, Canada
TWAEV	10 mg/m ³	Ontario, Canada
TWAEV	10 mg/m ³	Quebec, Canada
TWA	10 mg/m ³	USA

- **Appropriate engineering controls:**
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.
Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.
Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	Danger of explosion: Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	Explosion limits: Lower: 0.9 Vol %; Upper: 0.0 Vol %.
pH: Not available.	Decomposition temperature: Not available.
Melting point/freezing point: Not determined.	Vapor pressure at 20 °C: 0.1 hPa
Boiling point/Boiling range: 100 °C.	Density: Not determined.
Flash point: > 100 °C.	Relative density: Not determined.
Flammability (solid, gaseous): Not determined.	Vapor density: Not determined.
Ignition temperature: 400 °C.	Evaporation rate: Not determined.
Auto-igniting: Product is not self-igniting.	Solubility in / Miscibility with Water: Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion: May be harmful if swallowed.
Skin: May be harmful if absorbed through skin. May cause skin irritation.
Eyes: May cause eye irritation.
- **Signs and Symptoms of Exposure:**
Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 - Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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