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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
K316-380G-05	5 µg
K316-380G-10	10 µg

KAT7 (MYST2), Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog # K316-380G

Lot # Q2525-9

Product Description

Full-length recombinant human KAT7 (MYST2) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_007067.

Gene Aliases

KAT7; MYST2; HBO1; HBOA

Formulation

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

Storage and Stability

Store product at –70ºC. 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.

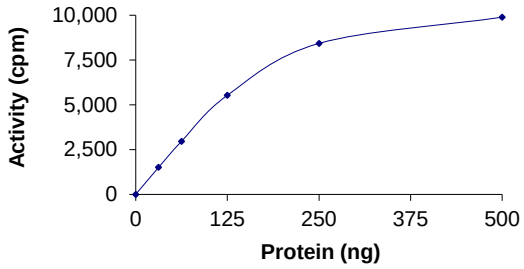
Scientific Background

KAT7 or K (lysine) acetyltransferase 5 is a signaling protein that belongs to the MYST family of histone acetyl transferases (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

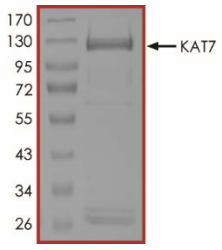
1. Sharma, M. et.al: Androgen receptor interacts with a novel MYST protein, HBO1. J. Biol. Chem. 275: 35200-35208, 2000.
2. Iizuka, M. et.al: Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. J. Biol. Chem. 274: 23027-23034, 1999.

Specific Activity



The specific activity of KAT7 (MYST2) was determined to be **0.5 nmol /min/mg** as per activity assay protocol.

Purity



The purity of KAT7 (MYST2) was determined to be **>90%** by densitometry.
Approx. MW **110kDa**.

KAT7 (MYST2), Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog #	K316-380G
Specific Activity	0.5 nmol/min/mg
Lot #	Q2525-9
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at –70ºC from date of shipment
Storage & Shipping	Store product at –70ºC. 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 Kinase (Catalog #: K316-380G)

Active KAT7 (MYST2) (0.1μg/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 (MYST2) for optimal results).

Acetyltransferase Dilution Buffer (Catalog#: A21-09)

Acetyltransferase Assay Buffer (Catalog #: A01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/μl BSA solution.

Acetyltransferase Assay Buffer (Catalog #: 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.

[^{3}H]-Acetyl-CoA solution

The [Acetyl ^{3}H]-CoA solution (0.1μCi/μl and 2.1μCi/nmol) in 10mM sodium acetate, pH 5.0 was purchased from PerkinElmer (Cat. # NET290250UC). The final concentration of Acetyl-CoA is 47.62 μM or 47.62 pmol/μl.

Substrate (Catalog #: H10-54N)

Histone H1 protein diluted in 50mM Tris-HCl, pH 7.5, and 150mM NaCl buffer to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [Acetyl ^{3}H]-CoA solution in shielded container in a designated radioactive working area.

Step 2. Thaw the Active KAT7, Acetyltransferase Assay Buffer, Substrate and Acetyltransferase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active KAT7 (MYST2) (Catalog #K316-380G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #H10-54N)
- **Component 3.** 5μl of Acetyltransferase Assay Buffer (Catalog #: A01-09)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5μl [Acetyl ^{3}H]-CoA solution bringing the final volume up to 25μl and incubate the mixture in a water bath at 30^{o}C for 30 minutes.

Step 6. After the 30 minute incubation period, terminate the reaction by spotting 20μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 50mM Na_2HPO_4, pH 9.0 solution with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [^{3}H]-Acetyl-CoA Specific Activity (SA) (cpm/nmol)

Specific activity (SA) = cpm for 5μl [Acetyl ^{3}H]-CoA / nmoles of Acetyl-CoA
5μl of a 47.62 μM Acetyl-CoA solution gives 142,000cpm
Therefore 142,000cpm / 5μl*47.62 pmol/μl = 596.39 cpm/pmol

Acetyltransferase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of [Acetyl ^{3}H]-CoA in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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