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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
K311-381G-05	5 µg
K311-381G-10	10 µg

KAT2A (GCN5), Active

Recombinant human protein expressed in Sf9 cells

Catalog # K311-381G
Lot # A1461-2

Product Description

Recombinant human KAT2A (GCN5) (323-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_021078.

Gene Aliases

GCN5; GCN5L2; HsGCN5; hGCN5; MGC102791; PCAF-b

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 –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.

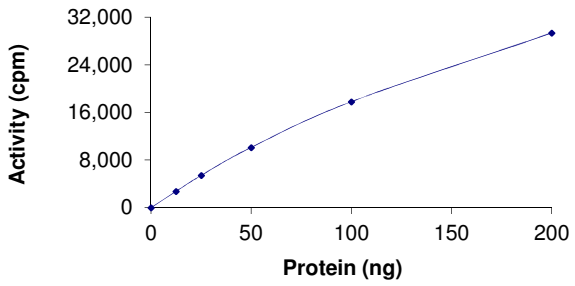
Scientific Background

KAT2A (K (lysine) acetyltransferase 2A) is a histone acetyltransferase (HAT) that functions primarily as a transcriptional activator which functions as a repressor of NF-kappa-B by promoting ubiquitination of the NF-kappa-B subunit RELA in a HAT-independent manner (1). KAT2A control chromosome stability by coordinating the ATR checkpoint and double-strand break processing with autophagy. KAT2A acetyltransferases have homologous sequences and enzymatic activities which are important for recognition of nucleosomal substrates (2).

References

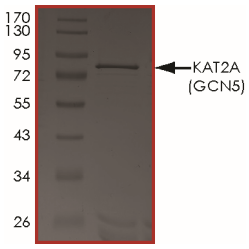
1. Mao, X. et.al: GCN5 is a required cofactor for a ubiquitin ligase that targets NF-kappa-B/RelA. Genes Dev. 23: 849-861, 2009.
2. Xu, W. et.al: Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates. Molec. Cell. Biol. 18: 5659-5669, 1998.

Specific Activity



The specific activity of KAT2A (GCN5) was determined to be **20 nmol /min/mg** as per activity assay protocol.

Purity



The purity of KAT2A (GCN5) was determined to be **>85%** by densitometry.
Approx. MW **82kDa**.

KAT2A (GCN5), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	K311-381G
Specific Activity	20 nmol/min/mg
Lot #	A1461-2
Purity	>85%
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 #: K311-381G)

Active KAT2A (GCN5) (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 KAT2A (GCN5) 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 #: H12-58)

Histone H3 Peptide (1-21) diluted in distilled H_2O 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 KAT2A, 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 KAT2A (GCN5) (Catalog #K311-381G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #H12-58)
- **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°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 acetyltransferase 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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