

KAT2A (GCN5), Active

Recombinant human protein expressed in Sf9 cells

Catalog # K311-381G

Lot # Y1078-2

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.

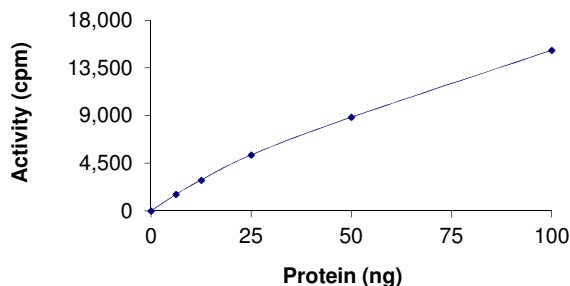
Scientific Background

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

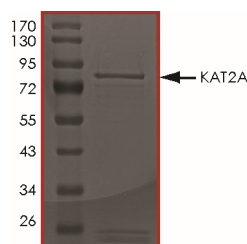
- Mao, X. et.al: GCN5 is a required cofactor for a ubiquitin ligase that targets NF-kappa-B/RelA. *Genes Dev.* 23: 849-861, 2009.
- Xu, W. et.al: Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates. *Molec. Cell. Biol.* 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 #	Y1078-2
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.

[³H]-Acetyl-CoA solution

The [Acetyl ³H]-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 H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [Acetyl ³H]-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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5µl [Acetyl ³H]-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 Na₂HPO₄, 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 [³H]-Acetyl-CoA Specific Activity (SA) (cpm/nmol)

Specific activity (SA) = cpm for 5µl [Acetyl ³H]-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 ³H]-CoA in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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