

## KAT2A (GCN5), Active

Recombinant human protein expressed in Sf9 cells

**Catalog # K311-381G**

Lot # G1153-3

### 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  $-70^{\circ}\text{C}$ . 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.

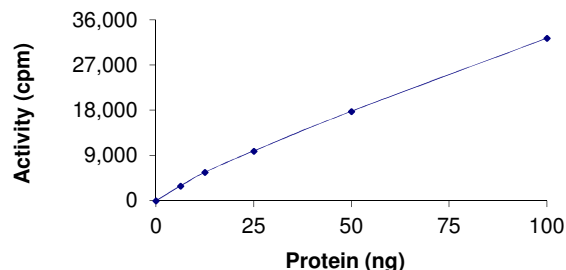
### Scientific Background

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

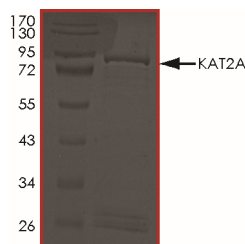
1. Mao, X. et.al: GCN5 is a required cofactor for an ubiquitin ligase that targets NF-kappa-B/RelA. *Genes Dev.* 23: 849-861, 2009.
2. Xu, W. et.al: Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates. *Molec. Cell. Biol.*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

### Specific Activity



The specific activity of KAT2A (GCN5) was determined to be **19 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	19 nmol/min/mg
Lot #	G1153-3
Purity	>85%
Concentration	0.05 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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) (0.05 $\mu$ g/ $\mu$ l) 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/ $\mu$ l 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.

### [<sup>3</sup>H]-Acetyl-CoA solution

The [Acetyl <sup>3</sup>H]-CoA solution (0.1 $\mu$ Ci/ $\mu$ l and 2.1 $\mu$ Ci/nmol) in 10mM sodium acetate, pH 5.0 was purchased from PerkinElmer (Cat. # NET290250UC). The final concentration of Acetyl-CoA is 47.62  $\mu$ M or 47.62 pmol/ $\mu$ l.

### Substrate (Catalog #: H12-58)

Histone H3 Peptide (1-21) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [Acetyl <sup>3</sup>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 20 $\mu$ l:
  - Component 1.** 10 $\mu$ l of diluted Active KAT2A (GCN5) (Catalog #K311-381G)
  - Component 2.** 5 $\mu$ l of 1mg/ml stock solution of substrate (Catalog #H12-58)
  - Component 3.** 5 $\mu$ l 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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5 $\mu$ l [Acetyl <sup>3</sup>H]-CoA solution bringing the final volume up to 25 $\mu$ l 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 20 $\mu$ l 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<sub>2</sub>HPO<sub>4</sub>, 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 [<sup>3</sup>H]-Acetyl-CoA Specific Activity (SA) (cpm/nmol)

Specific activity (SA) = cpm for 5 $\mu$ l [Acetyl <sup>3</sup>H]-CoA / nmoles of Acetyl-CoA  
5 $\mu$ l of a 47.62  $\mu$ M Acetyl-CoA solution gives 142,000cpm  
Therefore 142,000cpm / 5 $\mu$ l\*47.62 pmol/ $\mu$ l = 596.39 cpm/pmol

### Acetyltransferase Specific Activity (SA) (pmol/min/ $\mu$ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of [Acetyl <sup>3</sup>H]-CoA in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]

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