**COMT, Active**
Recombinant human protein expressed in Sf9 cells

**Catalog # C339-381G**
Lot # B2119-7

**Product Description**
Recombinant human COMT (27-271) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The COMT gene accession number is NM_000754.

**Gene Aliases**
HEL-S-98n

**Formulation**
Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM 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**
Catechol O-methyltransferase (COMT) is a major catabolic regulator of synaptic catecholamine neurotransmitters and it catalyzes the transfer of a methyl group to catecholamines and degrades dopamine, norepinephrine and epinephrine. The two forms of COMT [soluble COMT [S-COMT] and membrane-bound COMT [MB-COMT]] encoded by a single gene located on 22q11.2 have been identified. Activation of COMT affects the biological half-lives of certain neuroactive drugs.

**References**
3. http://www.uniprot.org/uniprot/P21964

**Specific Activity**
![Activity vs Protein graph]
The specific activity of COMT was determined to be 160 nmol/min/mg as per activity assay protocol.

**Purity**
The purity of COMT was determined to be >95% by densitometry, approx. MW 53 kDa.

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**Specific Activity**
160 nmol/min/mg

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**Purity**
>95%

**Concentration**
0.1 µg/µl

**Stability**
1 yr 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 Methyltransferase** (Catalog #: C339-381G)
Active COMT (0.1 µg/µl) diluted with Methyltransferase Reaction Buffer 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 COMT for optimal results).

**Methyltransferase Reaction Buffer**
Buffer components: 20mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 3 mM MgCl₂, 0.1 mg/ml BSA. Add 1mM DTT prior to use.

**MTase-Glo™ Methyltransferase Assay** (Promega, Catalog #: V7601)
S-Adenosyl-Methionine (SAM), 1mM
S-Adenosyl-Homocysteine (SAH), 15 µM
Methyltransferase-Glo™ Reagent, 10X
MTase-Glo™ Detection Solution, 1 bottle

**Substrate** (Sigma Cat. # H8502-10G)
Dopamine hydrochloride diluted in distilled H₂O to a final concentration of 1mM.

Assay Protocol

The COMT assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

**Step 1.** Thaw the active COMT and all Methyltransferase-Glo™ Assays kit reagents on ice.

**Step 2.** Prepare the following working solutions with Methyltransferase Reaction Buffer on ice:
- 2X final concentration of Active COMT (Catalog # C339-381G)
- 2X Substrate Cocktail: 40 µM of SAM and 40 µM of dopamine hydrochloride in water

**Step 3.** In a polystyrene 96-well plate, add the following components to bring the initial reaction volume to 20 µl:

- Component 1. 10 µl of 2X Substrate Cocktail
- Component 2. 10 µl of 2X Active COMT

**Note:** A blank control can be set up as outlined in step 3 by replacing the enzyme working solution with an equal volume of Reaction Buffer.

**Step 4.** Mix the reaction on an orbital shaker for 2 minutes. Seal the plate with a plate seal and incubate at 37°C for 60 minutes.

**Step 5.** Dilute 10X Methyltransferase-Glo™ Reagent with equal volume of nanopure water, and add 5 µl of the 5X Methyltransferase-Glo™ Reagent to all reaction wells.

**Step 6.** Mix on an orbital shaker for 2 minutes and then incubate at room temperature for 30 minutes.

**Step 7.** Add 25 µl of MTase-Glo™ Detection Solution to all reaction wells. Mix for 2 minutes and then incubate at room temperature for 30 minutes.

**Step 8.** Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).

**Step 9.** Using the SAH standard curve, determine the concentration of SAH produced (nM) and calculate the methyltransferase specific activity as outlined below. For a detailed protocol of how to determine SAH amount from RLUs, see MTase-Glo™ Methyltransferase Assay protocol at Promega’s website: www.promega.com/protocols

**Methyltransferase Specific Activity (SA) (nmol/min/mg)**

\[
SA = \frac{[SAH](nM) \times \text{Reaction Volume (µl)}}{\text{Reaction Time (min)} \times \text{Enzyme Amount (mg)}} \times 10^{-6}
\]

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