Recombinant Human Cytosolic Sulfotransferase 1E1/SULT1E1

Catalog Number: 5545-ST

DESCRIPTION

Source	*E. coli*-derived Asn2-Ile294, with an N-terminal 6-His tag Accession # P49888
N-terminal Sequence Analysis	His
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	35 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to 1-Napthol. The specific activity is >40 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Universal Sulfotransferase Activity Kit (Catalog # EA003)
- 10X Assay Buffer (supplied in kit): 500 mM Tris, 150 mM $MgCl_2$, pH 7.5
- Recombinant human SULT-1E1 (rhSULT-1E1) (Catalog # 5545-ST)
- 3'-Phosphoadenosine-5'-phosphosulfate (PAPS) (Catalog # ES019)
- 1-Naphthol (Sigma, Catalog # N1000), 10 mM in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer by diluting 10X stock 10-fold with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Universal Sulfotransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Prepare a reaction mixture containing 0.4 mM PAPS, 0.4 mM 1-Naphthol, and 0.02 mg/mL Coupling Phosphatase 3 in 1X Assay Buffer.
5. Dilute rhSULT-1E1 to 20 µg/mL in 1X Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
7. Load 25 µL of the 20 µg/mL rhSULT-1E1 into the plate. Include a Control containing 25µL of 1X Assay Buffer.
8. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
9. Seal plate and incubate at 37° C for 20 minutes.
10. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
11. Add 100 µL of deionized water to all wells. Mix briefly.
12. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate for 20 minutes at room temperature.
13. Read plate at 620 nm (absorbance) in endpoint mode.
14. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhSULT-1E1: 0.5 µg
- PAPS: 10 nmoles (0.2 mM)
- 1-Naphthol: 0.2 mM
- Coupling Phosphatase 3: 0.5 µg

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Cytosolic Sulfotransferases catalyze the sulfation of many hormones, neurotransmitters, drugs, and xenobiotic compounds. They are distinct from Golgi resident sulfotransferases by the absence of transmembrane domains and are located in the cytoplasm. SULT1E1 is widely known as an estrogen sulfotransferase and may control estrogen levels by converting free estradiol to its inactive sulfate conjugate (1). Known substrates of this enzyme also include dehydroepiandrosterone, pregnenolone, ethinylestradiol, equalenin, diethylstilbesterol, 4-nitrophenol, 1-napthol and resveratrol (2, 3). SULT1E1 activity is found in reproductive organs (4), intestine and liver (3, 5). The enzymatic activity of the recombinant human SULT1E1 is measured using a phosphatasecoupled assay (6).

References:

1. Gong, H. *et al*. (2008) Cancer Res. **68**:7386.
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4. Takase, Y. *et al*. (2007) The Prostate **67**:405.
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6. Prather, B. *et al*. (2012) Anal. Biochem. **423**:86.