Recombinant Human Cytosolic Sulfotransferase 1A3/SULT1A3

Catalog Number: 5829-ST

DESCRIPTION

Source	*E. coli*-derived Glu2-Leu295 with an N-terminal Met and 6-His tag Accession # P50224
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	35 kDa

SPECIFICATIONS

SDS-PAGE	36 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to 1-Napthol. The specific activity is >150 pmol/min/µg, as measured under the described conditions.
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, Glycerol and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 15 mM $MgCl_2$, pH 7.5
- Recombinant Human Sulfotransferase 1A3/SULT1A3 (rhSULT1A3) (Catalog # 5829-ST)
- 3'-Phosphoadenosine-5'-phosphosulfate/PAPS (Catalog # ES019)
- 1-Naphthol (Sigma, Catalog # N1000), 10 mM in deionized water
- Universal Sulfotransferase Activity Kit (Catalog # EA003)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. 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 Assay Buffer for a 100 µM stock.
2. Prepare a standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare a reaction mixture containing 0.4 mM PAPS, 0.4 mM 1-Naphthol, and 0.02 mg/mL Coupling Phosphatase 3 in Assay Buffer.
4. Dilute rhSULT1A3 to 10 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of the diluted rhSULT1A3 into the plate. Include a Substrate Blank containing 25 µL of Assay Buffer.
7. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
8. Cover the plate with a plate sealer and incubate at 37 °C for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. 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 or 4-parameter fitting and adjusted for Substrate Blank.

Final Assay Conditions

Per Reaction:

- rhSULT1A3: 0.25 µg
- PAPS: 10,000 pmol (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 are a family of Phase II drug-metabolizing enzymes that catalyze the sulfation of many endogenous and xenobiotic substrates (1-3). They have important roles in the metabolism of many endogenous compounds including steroids, bile acids, thyroid hormones and monoamine neurotransmitters. They are widely distributed throughout the body and serve to inactivate and increase water-solubility of xenobiotics and therapeutic drugs. They are distinct from Golgi resident sulfotransferases by lacking N-terminal signal-anchor domains and residing only in the cytoplasm. SULT1A3 catalyzes the sulfation of phenolic monoamines, such as dopamine, norepinephrine and serotonin, and phenolic and catecholic drugs (4, 5). SULT1A3 is also called catecholamine sulfotransferase. The enzymatic activity of the recombinant human SULT1A3 is measured using a phosphatasecoupled assay (6).

References:

1. Falany, C. N. (1997) FASEB J. **11**:206.
2. Gamage, N. U. *et al.* (2006) Toxicol. Sci. **90**:5.
3. Allali-Hassani, A. *et al.* (2007) PLoS Biol. **5**:e97.
4. Dooley, T.P. *et al.* (1994) Biochem. Biophys. Res. Commun. **205**:1325.
5. Salman, E.D. *et al.* (2009) Drug Metab. Dispos. **37**:706.
6. Prather, B. *et al.* (2012) Anal. Biochem. **423**:86.

PRODUCT SPECIFIC NOTICES

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