Recombinant Human Cytosolic Sulfotransferase 1C2/SULT1C2

Catalog Number: 7458-ST

DESCRIPTION

Source	*E. coli*-derived Ala2-Leu296, with N-terminal 6-His tag Accession # O00338
N-terminal Sequence Analysis	Inconclusive. Protein identity confirmed by mass spec, His tag confirmed by anti-His Western analysis.
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	34-37 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to 4-nitrophenol. The specific activity is >45 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	>80%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer (1X Phosphatase Buffer 3): 50 mM Tris, 15 mM $MgCl_2$, pH 7.5
- Recombinant Human Cytosolic Sulfotransferase 1C2/SULT1C2 (rhSULT1C2) (Catalog # 7458-ST)
- Coupling Enzyme: Recombinant Mouse IMPAD1 (Catalog # 7028-PD)
- Adenosine 3′-phosphate 5′-phosphosulfate lithium salt hydrate (PAPS) (Catalog # ES019), 1 mM stock in 5% ethanol, 95% deionized water
- 4-Nitrophenol (Sigma, Catalog # 241326), 50 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 Coupling Phosphatase 3 to 0.1 mg/mL in Assay Buffer.
2. Prepare reaction mixture by combining 50 µL of 0.1 mg/mL Coupling Phosphatase 3, 50 µL of 50 mM 4-Nitrophenol, 50 µL of 1 mM PAPS, and 100 µL of Assay Buffer. This is sufficient to assay 9 wells.
3. Dilute rhSULT1C2 to 40 µg/mL in Assay Buffer.
4. Dilute 1 mM Phosphate Standard by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
5. Continue 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 5.0 nmol per well.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
7. Load 25 µL of the 40 µg/mL rhSULT1C2 into the plate. Include a control containing 25 µL of 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 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 or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

- rhSULT1C2: 1.0 µg
- Coupling Phosphatase 3: 0.5 µg
- 4-Nitrophenol: 5 mM
- PAPS: 0.1 mM

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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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

Cytosolic sulfotransferases catalyze the sulfonation 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 (1, 2). SULT1C2 is mainly expressed in the gastrointestinal tract (stomach, duodenum, jejunum, ileum, colon, caecum and rectum), liver and kidneys, but not in the lungs (3). In contrast, SULT1C4, a sulfotransferase that is most closely related to SULT1C2 (4), is expressed at higher levels in fetal lung and kidney and at lower levels in fetal heart. So far, SULT1C2 is found to be active only on *p*-nitrophenol (3). The enzymatic activity of our recombinant human SULT1C2 was determined using a phosphatase-coupled assay (5).

References:

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2. Gamage, N. U. *et al.* (2006) Toxicol. Sci. **90**:5.
3. Hehonah, N. *et al.* (1999) Int J. Biochem. Cell. Biol. **31**:869.
4. Sakakibara, Y. *et al.* (1998) J. Biol. Chem. **273**:33929.
5. Prather, B. *et al.* (2012) Anal. Biochem. **423**:86.

PRODUCT SPECIFIC NOTICES

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449