Recombinant Human Arylsulfatase B/ARSB

Catalog Number: 4415-SU

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Ser37-Met533, with a C-terminal 10-His tag Accession # P15848
N-terminal Sequence Analysis	Ser37
Predicted Molecular Mass	57 kDa

SPECIFICATIONS

SDS-PAGE	80 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS). The specific activity is >3,000 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, pH 6.5
- Recombinant Human Arylsulfatase B/ARSB (rhARSB) (Catalog # 4415-SU)
- Substrate: 4-Nitrocatechol Sulfate (4-PNCS) (Sigma, Catalog # N-7251)
- NaOH (Sigma, Catalog # S-0899)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhARSB to 1 µg/mL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. Combine 75 µL of 1 µg/mL rhARSB and 75 µL of 2 mM Substrate. Include a Substrate Blank containing 75 µL Assay Buffer and 75 µL Substrate.
4. Incubate at 37 °C for 1 hour.
5. Stop reaction by adding 150 µL of 0.2 M NaOH.
6. Load 200 µL of reaction into a plate.
7. Read at 510 nm (absorbance) in endpoint mode.
8. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Abs* (OD) x Conversion Factor** (pmol/OD)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard P-Nitrocatechol (PNC) (Sigma, Catalog # N15553).

Final Assay Conditions

Per Well:

- rhARSB: 0.050 µg
- Substrate: 0.5 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

ARSB is a lysosomal enzyme of the sulfatase family that functions as an *N*-acetylgalactosamine-4-sulfatase (1). The enzyme is also active against chondroitin sulfate and dermatan sulfate (2). Deficiencies in ARSB activity are responsible for the lysosomal storage disease mucopolysaccharidosis Type VI, also known as Maroteaux-Lamy Syndrome (3).

References:

1. Peters, C. *et al.* (1990) J. Biol. Chem. **265**:3374.
2. O'Brien, J.S. *et al.* (1974) Biochem. Biophys. Res. Commun. **60**:1170.
3. Wicker, G. *et al.* (1991) J. Biol. Chem. **266**:21386.

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