

DESCRIPTION

Source Chinese Hamster Ovary cell line, CHO-derived
Gly17-Val525, with a C-terminal 10-His tag
Accession # Q3TYD4

N-terminal Sequence Analysis Gly17

Predicted Molecular Mass 57 kDa

SPECIFICATIONS

SDS-PAGE 65 kDa, reducing conditions

Activity Measured by its ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS).
The specific activity is > 1,500 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, 250 mM NaCl, pH 5.5
 - Recombinant Mouse Arylsulfatase G/ARSG (rmARSG) (Catalog # 4600-SU)
 - Substrate: 4-Nitrocatechol Sulfate (PNCS) (Sigma, Catalog # N-7251), 100 mM in deionized water
 - Sodium Hydroxide, 1 M stock in deionized water
 - 96-well Clear Plate (Costar, Catalog # 92592)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Dilute rmARSG to 1 μg/mL in Assay Buffer.
 2. Dilute Substrate to 2 mM in Assay Buffer.
 3. Mix 75 μL of 1 μg/mL of rmARSG and 75 μL of 2 mM of Substrate in duplicate. Create a Substrate Blank with 75 μL Assay Buffer and 75 μL Substrate in duplicate. Incubate at 37 °C for 1 hour.
 4. Stop reaction by adding 150 μL of 0.2 M sodium hydroxide to the reaction vials.
 5. Load into a clear 96-well plate 200 μL from each reaction vial.
 6. Read at 510 nm (absorbance) in endpoint mode.
 7. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

- Final Assay Conditions**
- Per Well:
- rmARSG: 0.05 μg
 - Substrate: 0.5 mM

PREPARATION AND STORAGE

Shipping The product is shipped with polar packs. 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

ARSG is a member of the aryl sulfatase family of enzymes with a high degree of amino acid sequence similarity to ARSA and ARSB. ARSG mRNA is expressed in a ubiquitous manner, being detected in most adult and embryonic tissues of the mouse (1).

References:

1. Ferrante, F. *et al.* (2002) *Eur. J. Hum. Genetics* **10**:813.