Recombinant Mouse Arylsulfatase A/ARSA

Catalog Number: 2858-SU

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ser18-Ser506, with a C-terminal 10-His tag Accession # Q9DC66
N-terminal Sequence Analysis	Ser18
Predicted Molecular Mass	54 kDa

SPECIFICATIONS

SDS-PAGE	60 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS). The specific activity is >100 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 Sodium Acetate, pH 5.0
- Recombinant Mouse Arylsulfatase A/ARSA (rmARSA) (Catalog # 2858-SU)
- Substrate: 4-Nitrocatechol Sulfate (PNCS) (Sigma, Catalog # N-7251), 100 mM stock in diH2O
- 0.2 M Sodium Hydroxide
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rmARSA to 20 µg/mL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. Combine in triplicate 75 µL of 20 µg/mL rmARSA and 75 µL of 2 mM Substrate. Include a Substrate Blank containing Assay Buffer in place of rmARSA.
4. Incubate reactions for 30 minutes at 37 °C.
5. Stop reactions by adding 150 µL 0.2 M sodium hydroxide to each for a final concentration of 0.1 M sodium hydroxide per reaction.
6. Load into a clear 96-well plate 200 µL from each reaction vial.
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 4-Nitrocatechol (PNC) (Sigma, Catalog # N15553).

Final Assay Conditions

Per Well:

- rmARSA: 1 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

As a member of the sulfatase family, arylsulfatase A is encoded by the ARSA gene and required for the lysosomal degradation of cerebroside-3-sulfate, a sphingolipid sulfate ester and a major constituent of the myelin sheet (1). ARSA deficiency results in metachromatic leukodystrophy (MLD), a lysosomal storage disease in the central and peripheral nervous systems with severe and progressive neurological symptoms (2). The deduced amino acid sequence of mouse ARSA consists of a signal peptide (residues 1-17) and a mature chain (residues 18-506) (3). Recombinant mouse ARSA corresponds to the mature chain and has sulfatase activity as described in Activity Assay Protocol.

References:

1. Lukatela, G. *et al.* (1998) Biochemistry **37**:3654.
2. Parenti, G. *et al.* J. (1997) Curr. Opin. Genet. & Dev. **7**:386.
3. Kreysing, J. *et al. (*1994) Genomics **19**:249.

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