Recombinant Human Arylsulfatase A/ARSA
Catalog Number: 2485-SU

DESCRIPTION
Source: Mouse myeloma cell line, NS0-derived Arg19-Ala507, with a C-terminal 10-His tag
Accession # AAH14210

N-terminal Sequence Analysis: Arg19
Predicted Molecular Mass: 53 kDa

SPECIFICATIONS
SDS-PAGE: 63 kDa, reducing conditions
Activity: Measured by its ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS).
The specific activity is >25 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 NaOAc, 0.5 M NaCl, pH 4.5
- Recombinant Human Arylsulfatase A/ARSA (rhARSA) (Catalog # 2485-SU)
- Substrate: 4-Nitrocatechol Sulfate (PNCS) (Sigma, Catalog # N-7251), 100 mM stock in dH2O
- Sodium Hydroxide (NaOH), 2.0 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 rhARSA to 20 μg/mL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. Mix 75 μL of 20 μg/mL of rhARSA and 75 μL of 2 mM of Substrate. As a Substrate Blank combine 75 μL Assay Buffer with 75 μL Substrate only. Prepare in triplicate.
4. Incubate at 37 °C for 1 hour.
5. Stop reaction by adding 150 μL of 0.2 M NaOH to reaction vials.
6. Load in a clear 96-well plate 200 μL from each reaction vial.
7. Read at 510 nm (absorbance) in endpoint mode.
8. Calculate specific activity:

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\text{Specific Activity (pmol/min/μg)} = \frac{\text{Adjusted Abs} \times \text{Conversion Factor} \times \text{Incubation time (min)} \times \text{amount of enzyme (μg)}}{\text{Conversion Factor} \times \text{time (min)} \times \text{amount of enzyme (μg)}}
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*Adjusted for Substrate Blank
**Derived using calibration standard 4-Nitrocatechol (PNC) (Sigma, Catalog # N15553).

Final Assay Conditions Per Well:
- rhARSA: 1 μg
- PNCS: 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, ARSA is required for the lysosomal degradation of cerebroside-3-sulfate, a sphingolipid sulfate ester and a major constituent of the myelin sheet (1). The 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 human ARSA consists of a signal peptide (residues 1-18) and a mature chain (residues 19-507) (3). Recombinant human ARSA corresponds to the mature chain and has sulfatase activity described above.

References: