Recombinant Human Enolase 2/Neuron-specific Enolase His-tag

Catalog Number: 10412-EN

DESCRIPTION

Source	*E. coli*-derived human Enolase 2/Neuron-specific Enolase protein Met1-Leu434 Accession # P09104.3 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	48 kDa

SPECIFICATIONS

SDS-PAGE	46 kDa, under reducing conditions
Activity	Measured by its ability to convert phosphoglyceric acid to phosphoenolpyruvate. The specific activity is >6,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl, KCl and MgSO4. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 30 mM $MgCl_2$, 200 mM KCl, pH 7.5
- Recombinant Human Enolase 2/Neuron-specific Enolase His-tag (rhENO-2) (Catalog # 10412-EN).
- Substrate: L-2-Phosphoglyceric acid (2-PG) (Sigma, Catalog # 19710), 100 mM stock in deionized water
- Recombinant Human PKM-2 (PKM-2) (Catalog # 7244-PK)
- Recombinant Human LDH-A (LDH-A) (Catalog # 9158-HA)
- Adenosine diphosphate (ADP) (Sigma, Catalog # A2754), 200 mM stock in deionized water
- β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (β-NADH) (Sigma, Catalog # N8129), 20 mM stock in 0.1 M Sodium Borate, pH 9.0
- 96-well clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare Reaction Mixture containing 20 µg/mL rhPKM-2, 5 µg/mL rhLDH-A, 14 mM ADP, 800 µM β-NADH, and 8 mM 2-PG in Assay Buffer.
2. Incubate Reaction Mixture at room temperature for 5 minutes.
3. Dilute rhENO-2 to 1 µg/mL in Assay Buffer.
4. In a plate, load 50 µL of 1 µg/mL rhENO-2, and start the reaction by adding 50 µL of Reaction Mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Reaction Mixture.
5. Read at an absorbance of 340 nm in kinetic mode for 10 minutes with a lag time of 3 minutes.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol x (-1)}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhENO-2: 0.05 µg
- 2-PG: 4 mM
- ADP: 7 mM
- β-NADH: 400 µM

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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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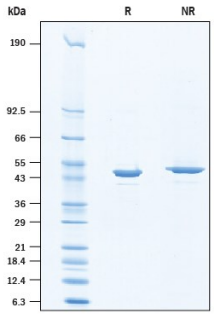
PREPARATION AND STORAGE

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.

DATA

Binding Activity



2 µg/lane of Recombinant Human Enolase 2/Neuron-specific Enolase His-tag (Catalog # 10412-EN) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 46 kDa under reducing conditions.

BACKGROUND

Neuron specific enolase (NSE), also known as ENO2 or gamma-enolase, is a dimeric, Mg^{2+}-dependent enzyme that catalyzes the dehydration of 2-phospho-D glycate (PGA) to phosphoenolpyruvate (PEP) in the glycolytic pathway and catalyzes the reverse reaction in gluconeogenesis. There are three major isozymes of enolase expressed in selective vertebrate tissues from separate genes: alpha (ENO1), beta (ENO3), and gamma (ENO2). NSE is a highly expressed, specific neuron isozyme (1) making it a useful marker for tumors derived from neuronal cells. Neuron-specific enolase is implicated as a diagnostic and prognostic marker in numerous diseases including early small cell lung cancer (2), prostate cancer (3), multiple myeloma (4), traumatic brain injury (5), acute spinal cord injury (6), acute ischemic stroke (7), and post-concussion symptoms (8). NSE expression and activity are increased in neuronal and glial activation and injury (9), risk factors implicated in neurodegenerative disease. Elevation of NSE promotes glycolysis, proliferation, activation and migration through its C-terminus to activate PI3K and MAPK signal transduction pathways (6, 10) while inhibition of enolase has been shown to attenuate inflammatory events (11, 12). NSE can be regulated through cleavage of the C-termini by cathepsin X (13, 14) or inhibited directly by antibiotic SF2312 (15). Inhibition has been proposed as a therapeutic strategy in cancer (16).

References:

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