Recombinant Human Kynureninase

Catalog Number: 4887-KH

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Met1-Asn465, with a C-terminal 10-His tag Accession # NP_003928
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	54 kDa

SPECIFICATIONS

SDS-PAGE	51 kDa, reducing conditions
Activity	Measured by its ability to oxidize 3-hydroxy kynurenine. The specific activity is > 75 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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.05% (w/v) Brij-35, 5 µM Pyridoxal Phosphate (Sigma, Catalog # P9255), pH 8.0
- Recombinant Human Kynureninase (rhKYNU) (Catalog # 4887-KH)
- Substrate: 3-hydroxy-DL-kynurenine (3-HK) (Sigma, Cat. # H1771), 10 mM in 50% DMSO, 50% deionized water
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhKYNU to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Load in a black well plate 50 µL of 2 ng/µL rhKYNU, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL 200 µM Substrate.
4. Read at excitation and emission wavelengths of 315 nm and 415 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 3-hydroxyanthranilinic acid (Sigma, Catalog # H9391).

Final Assay Conditions

Per Well:

- rhKYNU: 0.1 µg
- Substrate: 100 µM

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

Kynureninase is a pyridoxal-5 (-phosphate-dependent enzyme that catalyzes the hydrolytic cleavage of the amino acids L-kynurenine and L-3-hydroxykynurenine to give either anthranilic acid or 3-hydroxyanthranilic acid and alanine (1). The enzyme is a member of the “kynurenine pathway” enzymes, through which the majority of dietary tryptophan is degraded in the liver, and is involved in the *de novo* biosynthesis of NAD^+ (2, 3). Kynurenine pathway genes are expressed in immune system cells such as macrophages and microglia. During inflammatory responses, the kynurenine pathway in these cells produces quinolinic acid (QA) and not NAD^+. QA excites neurons via the activation of NMDA (N-methyl-D-aspartate) receptors resulting in neuronal damage. The tissue-damaging process has been demonstrated in AIDS-related dementia complex, Alzheimer’s, stroke, epilepsy, and Huntington’s disease. Because Kynureninase is one of the key enzymes of QA production, its inhibitors may be useful for the treatment of neurological disorders. The recombinant Kynureninase has been shown to possess specificity for 3-hydroxykynurenine over kynurenine (4, 5).

References:

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