Recombinant Human IDO2
Catalog Number: 9967-AO

DESCRIPTION

Source
E. coli-derived human IDO2 protein
Glu15-Gly420, with an N-terminal Met and C-terminal 6-His tag
Accession # Q6ZQW0-1

N-terminal Sequence Analysis
Met

Predicted Molecular Mass
46 kDa

SPECIFICATIONS

SDS-PAGE
41 kDa, reducing conditions

Activity
Measured by its ability to oxidize L-tryptophan to N-formyl-kynurenine.
The specific activity is >15 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 Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Assay Buffer: 50 mM Tris, 20% Glycerol, pH 7.5
- 0.405 M Tris, pH 8.0
- Recombinant Human IDO2 (rhIDO2) (Catalog # 9967-AO)
- Ascorbic Acid (Sigma, Catalog # 255564), 500 mM stock in deionized water
- L-Tryptophan (Sigma, Catalog # T0254), 40 mM stock in deionized water
- Catalase (Sigma, Catalog # C30), 100,000 units/mL stock diluted in 50 mM MES, pH 6.5
- Methylene Blue (Sigma, Catalog # 28514), 10 mM stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay
1. Prepare the Substrate Mixture.
   a. Dilute Ascorbic Acid to 80 mM in 0.405 M Tris, pH 8.0
   b. Prepare a mixture of 9000 units/mL Catalase and 40 µM Methylene Blue in Assay Buffer.
   c. Mix equal volumes of 1a and 1b for final concentrations of 40 mM Ascorbic Acid, 4500 units/mL Catalase and 20 µM Methylene Blue.
2. Dilute rhIDO2 to 160 ng/µL in Assay Buffer.
3. Load 25 µL of 160 ng/µL of rhIDO2-2 to a clear plate, and start the reaction by adding 25 µL of 40 mM L-Tryptophan, followed by 50 µL of Substrate Mixture. Include a Substrate Blank containing 25 µL of Assay Buffer, 25 µL of L-Tryptophan and 50 µL of Substrate Mixture.
4. Read in kinetic mode for 5 minutes at an absorbance of 321 nm.
5. Calculate specific activity:
   \[
   \text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\max}^* \times (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol ext. coeff}^{**} \text{ (M}^{-1}\text{cm}^{-1}) \times \text{path corr.*** (cm)} \times \text{amount of enzyme (µg)}}
   \]
   
   *Adjusted for Substrate Blank
   **Using the extinction coefficient 3750 M}^{-1}\text{cm}^{-1}
   ***Using the path correction 0.32 cm

Final Assay Conditions
Per Well:
- rhIDO2: 4.0 µg
- Ascorbic Acid: 20 mM
- L-Tryptophan: 10 mM
- Catalase: 225 units
- Methylene Blue: 10 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.

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Enzyme Activity

Recombinant Human IDO2 (Catalog # 9967-AO) is measured by its ability to oxidize L-tryptophan to N-formyl-kynurenine. The activity (orange) is approximately 4-fold greater than the competitor's IDO2 (green).

SDS-PAGE

1 μg lane of Recombinant Human IDO2 was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silverstaining, showing a band at 41 kDa.

BACKGROUND

Indoleamine 2,3-dioxygenase (IDO2) is a 47 kDa heme-containing cytosolic dioxygenase. Human IDO2 shares 64% aa sequence identity with mouse IDO2. IDO2 is one of three dioxygenases capable of catalyzing the first and rate-limiting step of the L-kynurenine pathway (KP): oxidative cleavage of the essential amino acid L-tryptophan to form N-formyl kynurenine (1). Of these proteins, IDO1 and IDO2 are both related, monomeric enzymes but share only 38% aa sequence identity. The IDO isoforms are not functionally redundant. Although expression of IDO2 has been upregulated in some cancers (2,3), IDO2 expression is generally restricted to the liver, kidney, brain, and certain immune cell types unlike the more ubiquitously expressed indoleamine 2,3-dioxygenase (IDO) (1). Differential inhibition of IDO1 and IDO2 is observed with several molecules (4,5). IDO2 has significantly lower tryptophan catabolic activity than IDO1 and TDO2 (4, 6-8) suggesting it does not play a significant physiological role in the KP. Instead, IDO2 may have an alternative functional role: either non-enzymatic or utilizing a more physiologically relevant substrate (5,7). IDO2 function operates as a pro-inflammatory mediator in autoimmune inflammatory disorders (8, 9-11). It is a candidate for co-therapeutic targeting for treatment in these diseases (10-11).

References: