Recombinant Mouse TDO2 His-tag

Catalog Number: 10001-TD

DESCRIPTION

Source	*E. coli*-derived mouse TDO2 protein Val18-Phe388 Accession # P48776 with a C terminal 6-His tag
N-terminal Sequence Analysis	Val18
Predicted Molecular Mass	45 kDa

SPECIFICATIONS

SDS-PAGE	39 & 75 kDa, reducing conditions
Activity	Measured by its ability to oxidize L-tryptophan to N-formyl-kynurenine. The specific activity is >500 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	>90%, 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 MES, pH 6.5
- 0.405 M Tris, pH 8.0
- Recombinant Mouse TDO2 (rmTDO2) (Catalog # 10001-TD)
- 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 in Assay Buffer
- 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 Substrate Mixtures.
   a. Dilute L-Tryptophan to 8 mM in Assay Buffer
   b. Dilute Ascorbic acid to 80 mM in 0.405 M Tris, pH 8.0.
   c. Prepare a mixture of 9000 Units/mL catalase, and 40 µM Methylene Blue in Assay Buffer.
   d. Mix equal volumes of 1b and 1c for final concentrations of 40 mM Ascorbic Acid, 4500 units/mL Catalase, and 20 µM Methylene Blue.
2. Dilute rmTDO2 to 40 ng/µL in Assay Buffer.
3. Load 25 µL of 40 ng/µL rmTDO2 to clear plate, and start the reaction by adding 25 µL of 8 mM L-Tryptophan followed by 50 µL of Mixture 1d. Include a Substrate Blank containing 25 µL Assay Buffer, 25 µL 8 mM L-Tryptophan and 50 µL of Mixture 1d.
4. Read plate in kinetic mode for 5 minutes at an absorbance of 321 nm.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank.
**Using the extinction coefficient 3750 M-1cm-1.
***Using the path correction 0.32 cm.
Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rmTDO2: 1.0 µg
- Ascorbic Acid: 20 mM
- L-Tryptophan: 2 mM
- Catalase: 225 units
- Methylene Blue: 10 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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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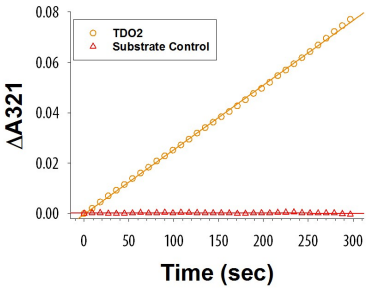
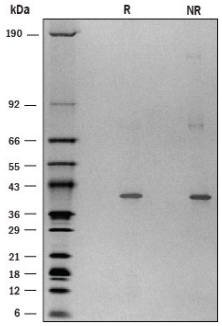
DATA

Enzyme Activity



Recombinant Mouse TDO2 (Catalog # 10001-TD) is measured by its ability to oxidize L-tryptophan to N-formyl-kynurenine.

SDS-PAGE



1 µg/lane of Recombinant Mouse TDO2 was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing bands at 39 kDa and 75 kDa under R conditions.

BACKGROUND

Tryptophan 2,3-dioxygenase (TDO2), a heme-containing cytosolic dioxygenase, forms a homo-tetrameric active molecule of approximately 190 kDa composed of 48 kDa monomers (1, 2). Mouse TDO2 shares 89% aa sequence identity with human TDO2. TDO2 is one of three proteins 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 (3). TDO2 is a cytosolic protein typically localized to the liver and brain, unlike the more ubiquitously expressed indoleamine 2,3-dioxygenase (IDO), yet it is responsible for ~90% of the primary route of catabolism of tryptophan through the KP (3). TDO2 is upregulated in extrahepatic tumors (4-6) and is consequently a target in cancer immunotherapy (7). TDO2 is a therapeutic target in brain disease such as schizophrenia, Alzheimers disease, multiple sclerosis and glioma (8-11) due to its role in the regulation of levels of critical biologically active downstream KP metabolites (3). Polymorphisms in the TDO2 gene have been implicated for a role in behavioural responses and autism (12,13).

References:

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