Active Indoleamine-2,3-Dioxygenase (IDO) Instruction Manual

SBPB237Hu01

Homo sapiens (Human)

Buffer Formulation	20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01%skl, 5% Trehalose and Proclin300.
Traits	Freeze-dried powder
Purity	> 97%
Isoelectric Point	6.8
Applications	Cell culture; Activity Assays.

ACTIVITY TEST

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

*Adjusted for Substrate Blank
**Using the extinction coefficient 3750 $M^{-1}cm^{-1}$
***Using the path correction 1 cm

IDO (Indoleamine 2,3-dioxygenase 1) is a heme enzyme that catalyzes the first and rate-limiting step in tryptophan catabolism to N-formyl-kynurenine. This enzyme acts on multiple tryptophan substrates including D-tryptophan, L-tryptophan, 5-hydroxy-tryptophan, tryptamine, and serotonin. Thus, bioactivity of recombinant human IDO was measured through its ability to oxidize L-tryptophan to N-formyl-kynurenine, using Methylene Blue as indicator. The reaction was performed in 50 mM MES, pH 6.5 (Assay Buffer), initiated by addition 50 µL of various concentrations of IDO (diluted by Assay Buffer) to 50 µL substrate mixture of 800 µM L-tryptophan, 9000 units/mL catalase（RPC418Hu05）, and 40 µM Methyene Blue in assay buffer with equal volume of 80 mM ascorbic acid in 0.405 M Tris, pH 8.0. The final well serves as a negative control with no IDO, replaced with 50µL assay buffer. The absorbance was read in 321 nm in kinetic mode for 5 minutes. The result indicated that recombinant human IDO can oxidize L-tryptophan, the specific activity is 10581 pmol/min/µg.

USAGE

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

Image

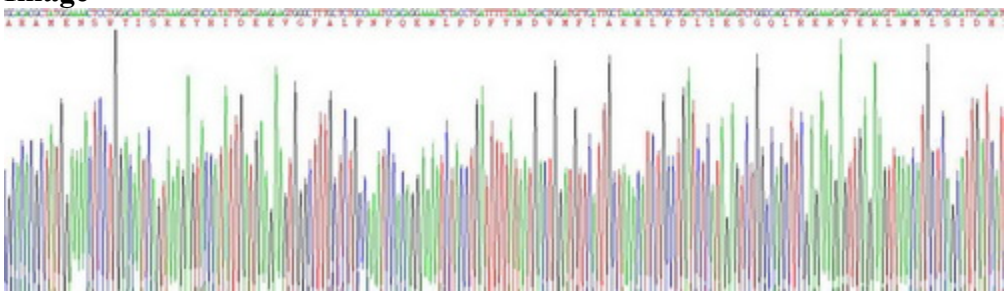

Figure. SDS-PAGE; Sample: Active recombinant IDO, Human.

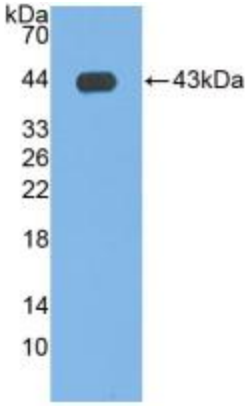

Figure. Western Blot; Sample: Recombinant IDO, Human.

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.