**DESCRIPTION**

**Source**  
E. coli-derived

<table>
<thead>
<tr>
<th>N-terminus</th>
<th>C-terminus</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>HHHHHH</td>
</tr>
</tbody>
</table>

**Human IDO**  
(Ala2-Gly403)  
Accession # P14902

**N-terminal Sequence Analysis**  
Met

**Structure / Form**  
Monomer

**Predicted Molecular Mass**  
46 kDa

**SPECIFICATIONS**

**SDS-PAGE**  
42 kDa, reducing conditions

**Activity**  
Measured by its ability to oxidize L-tryptophan to N-formylkynurenine.  
The specific activity is >500 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 Sodium Acetate, 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 Human Indoleamine 2,3-dioxygenase/IDO (rhIDO) (Catalog # 6030-AO)
- Ascorbic Acid (Sigma, Catalog # 255564), 500 mM stock in deionized water
- L-Tryptophan (Sigma, Catalog # T0254), 10 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 (Costar, Catalog # 92592)
- 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 800 μM L-Tryptophan, 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, 400 μM L-Tryptophan, 4500 units/mL Catalase, and 20 μM Methylene Blue.
2. Dilute rhIDO to 16 ng/μL in Assay Buffer.
3. Load into a plate 50 μL of 16 ng/μL rhIDO, and start the reaction by adding 50 μL of Substrate Mixture.
4. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of Substrate Mixture.
5. Read at 321 nm in kinetic mode for 5 minutes.

Specific Activity (pmol/min/μg) = __________ Adjusted Vmax * (OD/min) x well volume (L) x 10^{12} pmol/mol

ext. coeff** (M^{-1}cm^{-1}) x path corr.*** (cm) x amount of enzyme (μg)

*Adjusted for Substrate Blank  
**Using the extinction coefficient 3750 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:

- rhIDO: 0.800 μg
- Ascorbic Acid: 20 mM
- L-Tryptophan: 200 μM
- 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

---

**Rev. 2/6/2018 Page 1 of 2**
Indoleamine 2,3-dioxygenase (IDO) is a heme-containing intracellular dioxygenase catalyzing the degradation of the essential amino acid L-tryptophan to N-formylkynurenine (1). This degradation is the first and rate-limiting step of the L-kynurenine pathway (2). IDO is widely expressed in dendritic cells, macrophages, microglia, eosinophils, fibroblasts, endothelial cells, and most tumor cells. In immune cells, its expression is mainly induced by cytokines such as IFN-γ, IFN-α, IFN-β, and IL-10. IDO has an antimicrobial function due to its decreasing the availability of the essential amino acid tryptophan in inflammatory environments (3). Recent studies have demonstrated that IDO induces immunosuppression during infection, pregnancy, transplantation, autoimmunity, and neoplasia (3-5).

References: