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Data Sheet

TDO Inhibitor Screening Assay Kit

Catalog # 72023

BACKGROUND: L-tryptophan (L-Trp) is an essential amino acid necessary for protein synthesis in mammalian cells, and the L-Trp to kynurenine (Kyn) pathway is firmly established as a key regulator of innate and adaptive immunity. Catabolism of L-Trp to Kyn maintains an immunosuppressive microenvironment by starving immune cells of L-Trp and releasing degradation products of L-Trp that have immunosuppressive functions. Tryptophan 2,3 dioxygenase (TDO) is upregulated in many tumors, providing cancer cells with an avenue for immune evasion.

DESCRIPTION: The *TDO Inhibitor Screening Assay Kit* is designed to measure TDO enzyme inhibition. The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 100 reactions. The *TDO Inhibitor Screening Assay Kit* is simple to use. Inhibitor and enzyme are added to a sample containing L-Trp substrate. After a room temperature incubation, activity is measured by measuring the absorption of reaction product at λ = 320-325 nm.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71195	TDO, His-tag*	50 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
73005	TDO Reaction Solution	2 x 10 ml	-80°C	
73006	1x TDO Assay Buffer	1 ml	-80°C	
79965	UV transparent 96-well plate	1	Room Temp.	

**The concentration of TDO is lot-specific and will be indicated on the tube containing the enzyme.*

MATERIALS REQUIRED BUT NOT SUPPLIED:
Spectrophotometer capable of measuring absorbance at λ = 320-325 nm.
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for the study of TDO enzymology, screening inhibitors, and selectivity profiling.

CONTRAINDICATIONS: DMSO >0.5%, strong acids or bases, ionic detergents, and high salt

STABILITY: Stable at least 6 months from date of receipt, when stored as directed. Kit components require different storage conditions. Be sure to store each component at the proper temperature upon arrival.

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REFERENCE(S):

1. Li, J.S., *et al. Arch Insect Biochem Physiol*. 2007 Feb; **64(2):** 74–87

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

1) Thaw **TDO Reaction Solution** and aliquot 180 µl into each well. *Note: **TDO Reaction Solution** may contain a precipitate after thawing. Please ensure the mixture is fully solubilized before aliquoting by mixing thoroughly. Do not vortex.*

2) Add 10 µl of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor." For the "Positive Control" and "Blank," add 10 µl of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.

3) Thaw **TDO, His-tag**on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **TDO, His-tag** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **TDO,** **His-tag** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **TDO, His-tag** in **1x TDO Assay Buffer** at 50 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	**Positive Control**	**Test Inhibitor**
Reaction Solution	180 µl	180 µl	180 µl
Test Inhibitor	–	–	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	–
1x TDO Assay Buffer	10 µl	–	–
TDO**, His-tag** (50 ng/µl)	–	10 µl	10 µl
Total	**200 µl**	**200 µl**	**200 µl**

5) Add 10 µl of **1x TDO Assay Buffer** to the well designated "Blank."

6) Initiate reaction by adding 10 µl of diluted **TDO, His-tag** prepared as described above to the wells labeled "Positive Control," and "Test Inhibitor." Incubate at room temperature for 90 minutes.

7) Measure absorption at λ=320-325 nm.

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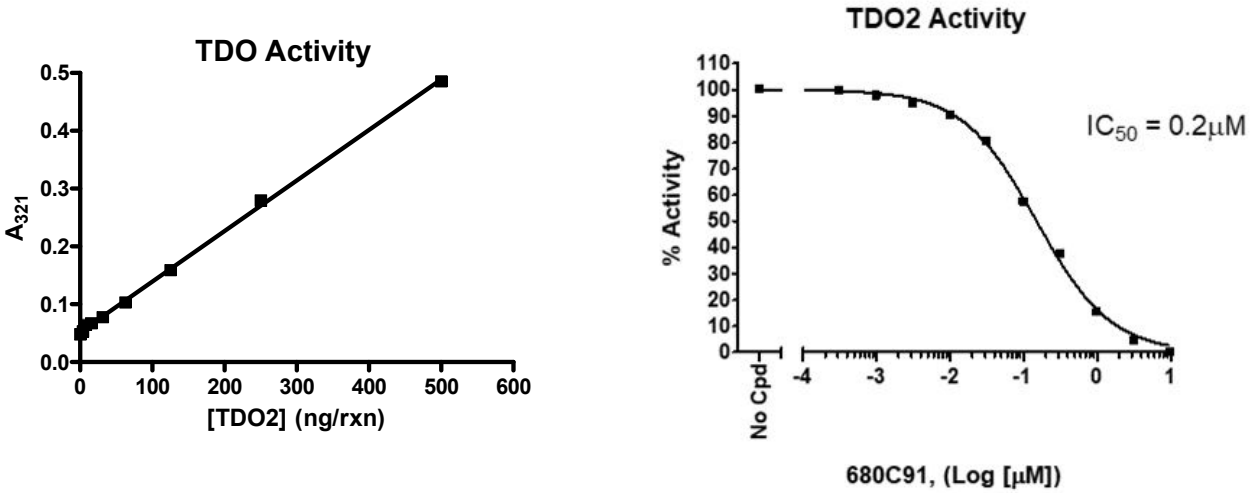
EXAMPLE OF ASSAY RESULTS:



TDO activity (left) and inhibition (right), measured using the TDO Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72023. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

Product	Catalog #	Size
TDO, His-tag	71195	50 µg
IDO1, His-tag	71182	50 µg
IDO2, His-tag	71194	200 µg
IDO1 Inhibitor Screening Assay Kit	72021	96 rxns
IDO2 Inhibitor Screening Assay Kit	72022	96 rxns
IDO1 Cell-Based Assay Kit	72031	100 rxns
TDO Cell-Based Assay Kit	72033	100 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Colorimetric Kit	72019	96 rxns
PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Assay Kit	72004	96 rxns
PD-1[Biotinylated]:PD-L1 Inhibitor Screening Assay Kit	72005	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Assay Kit	72006	96 rxns
CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72007	96 rxns
BTLA:HVEM[Biotinylated] Inhibitor Screening Assay Kit	72008	96 rxns
CTLA4:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72009	96 rxns
NLG919	27337-1	10 mg
NLG919	27337-2	50 mg
INCB024360	27338-1	10 mg