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## Data Sheet

### ***IDO1 Inhibitor Screening Assay Kit***

Catalog # 72021

**DESCRIPTION:** The *IDO1 Inhibitor Screening Assay Kit* is designed to measure IDO1 enzyme inhibition. The kit comes in a convenient format with enough reaction solution and enzyme to perform a total of 100 reactions. The *IDO1 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 determined by measuring the absorption of reaction product at  $\lambda=320 - 325$  nm.

**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. Indoleamine 2,3-dioxygenases (IDO1 & IDO2), two of the rate limiting enzymes in this pathway, are upregulated in many tumors, providing cancer cells with an avenue for immune evasion.

#### **COMPONENTS:**

Catalog #	Component	Amount	Storage	
71182	IDO1 His-Tag	40 $\mu$ g	-80°C	<b>Avoid freeze/ thaw cycles!</b>
73001	IDO1 Reaction Solution	2 x 10 ml	-80°C	
73002	1x IDO1 Assay Buffer	1 ml	-80°C	
79965	UV transparent 96-well plate	1	Room Temp.	

#### **MATERIALS REQUIRED BUT NOT SUPPLIED:**

Spectrophotometer capable of measuring absorbance at  $\lambda=320 - 325$  nm

Adjustable micropipettor and sterile tips

**APPLICATIONS:** Useful for the study of IDO1 enzymology, screening inhibitors, and selectivity profiling.

#### **CONTRAINDICATIONS:**

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

**STABILITY:** At least one year from date of receipt when stored as directed.

#### **REFERENCE(S):**

1. Liu, X., *et al.*, *Blood*. 2010; **115(17)**: 3520-3530.
2. Seegers, N., *et al.* *J Biomol Screen*. 2014; **19(9)**: 1266-74.

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### ASSAY PROTOCOL:

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

#### Step 1:

- 1) Thaw **IDO1 Reaction Solution** and aliquot 180  $\mu$ l into each well. *Note: **IDO1 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  $\mu$ l of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 10  $\mu$ l of the same solution without inhibitor (inhibitor buffer). *Note: Keep the DMSO concentration below 0.5%.*
- 3) Thaw **IDO1 His-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **IDO1 His-Tag** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **IDO1 His-Tag** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 4) Dilute **IDO1 His-Tag** in **1x IDO1 Assay Buffer** at 40 ng/ $\mu$ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
IDO1 Reaction Solution	180 $\mu$ l	180 $\mu$ l	180 $\mu$ l
Test Inhibitor	-	-	10 $\mu$ l
Inhibitor buffer (no inhibitor)	10 $\mu$ l	10 $\mu$ l	-
1x IDO1 Assay Buffer	10 $\mu$ l	-	-
IDO1 (40 ng/ $\mu$ l)	-	10 $\mu$ l	10 $\mu$ l
<b>Total</b>	<b>200 <math>\mu</math>l</b>	<b>200 <math>\mu</math>l</b>	<b>200 <math>\mu</math>l</b>

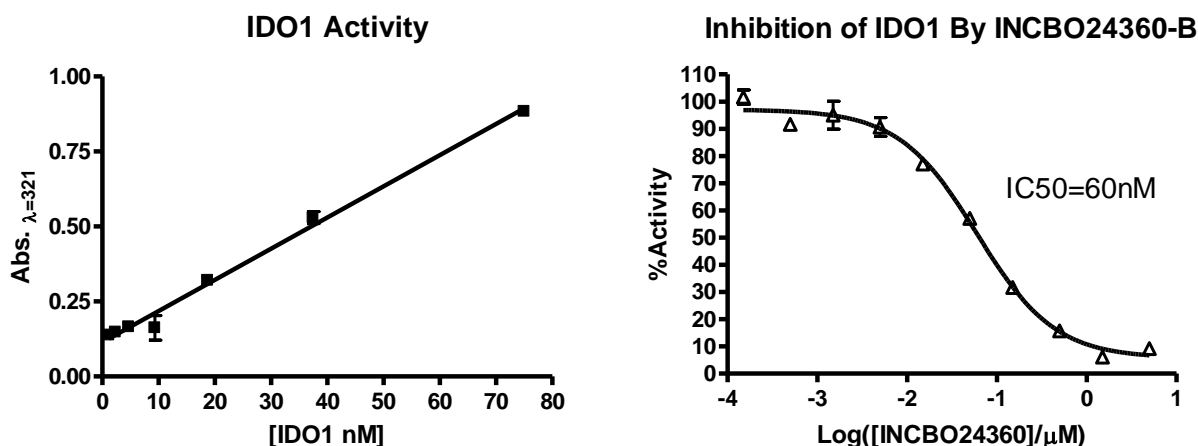
- 5) Add 10  $\mu$ l of **1x IDO1 Assay Buffer** to the well designated "Blank."
- 6) Initiate reaction by adding 10  $\mu$ l of diluted **IDO1 His-Tag** prepared as described above to the wells labeled "Positive Control" and "Test Inhibitor." Incubate at room temperature for 3 hours.
- 7) Measure absorption at  $\lambda=320 - 325$  nm.

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**EXAMPLE OF ASSAY RESULTS:**



IDO1 activity (right) and IDO1 inhibition (left), measured using the IDO1 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72021. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

**RELATED PRODUCTS:**

<u>Product</u>	<u>Catalog #</u>	<u>Size</u>
Human IDO1, His-tag	71182	50 $\mu$ g
Human IDO2, His-tag	71194	200 $\mu$ g
Human TDO, His-tag	71195	50 $\mu$ g
Human IDO2 Inhibitor Screening Assay Kit	72022	96 rxns
Human TDO Inhibitor Screening Assay Kit	72023	96 rxns
Human IDO1 Cell-Based Assay Kit	72031	100 rxns
Human TDO Cell-Based Assay Kit	72033	100 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
INCB024360	27338-2	100 mg

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