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## Data Sheet ***IDO2 Inhibitor Screening Assay Kit*** Catalog # 72022

**DESCRIPTION:** The *IDO2 Inhibitor Screening Assay Kit* is designed to measure IDO2 enzyme inhibition. The kit comes in a convenient format with enough reaction solution and enzyme to perform a total of 100 reactions. The *IDO2 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	
71194	IDO2 His-Tag	400 $\mu$ g	-80°C	<b>(Avoid freeze/ thaw cycles!)</b>
73003	IDO2 Reaction Solution	2 x 10 ml	-80°C	
73004	1x IDO2 Assay Buffer	1 ml	-80°C	
	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 IDO2 enzymology, screening inhibitors, and selectivity profiling.

### CONTRAINDICATIONS:

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

**STABILITY:** At least 6 months from date of receipt when stored as directed.

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#### 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.

#### ASSAY PROTOCOL:

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

#### Step 1:

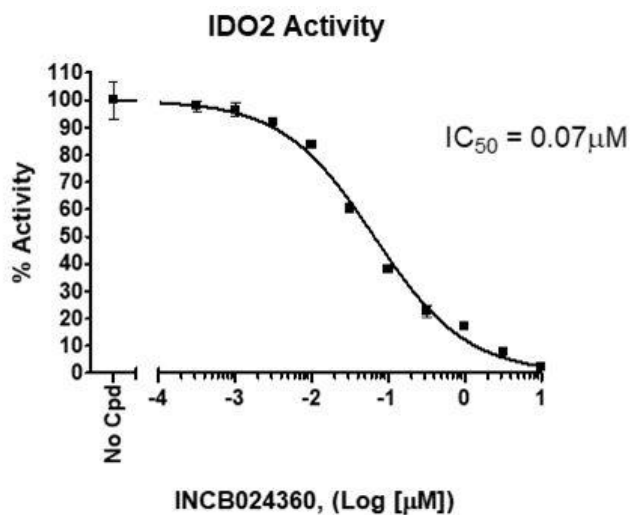
- 1) Thaw **IDO2 Reaction Solution** and aliquot 180  $\mu$ l into each well.
- 2) Add 10  $\mu$ l of inhibitor solution (containing no more than 10% DMSO) to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 10  $\mu$ l of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.
- 3) Thaw **IDO2 His-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **IDO2 His-Tag** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **IDO2 His-Tag** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
- 4) Dilute **IDO2 His-Tag** in **1x IDO2 Assay Buffer** at 400 ng/ $\mu$ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
IDO2 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 IDO2 Buffer	10 $\mu$ l	-	-
IDO2 (400 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 IDO2 Assay Buffer** to the well designated "Blank".
- 6) Initiate reaction by adding 10  $\mu$ l of diluted **IDO2 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:**

IDO2 activity (right) and measured using the IDO2 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72022. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com).*

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**RELATED PRODUCTS:**

<b><u>Product</u></b>	<b><u>Catalog #</u></b>	<b><u>Size</u></b>
IDO1, His-tag	71182	50 µg
IDO2, His-tag	71194	200 µg
TDO, His-tag	71195	50 µg
IDO1 Inhibitor Screening Assay Kit	72021	96 rxns
TDO Inhibitor Screening Assay Kit	72023	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 Assay 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

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