

Data Sheet

Mouse IDO2 Inhibitor Screening Assay Kit

Catalog # 72042

DESCRIPTION: The *IDO2 Inhibitor Screening Assay Kit* is designed to measure mouse IDO2 enzyme inhibition. The kit comes in a convenient format with enough reaction solution and enzyme to perform a total of 100 reactions. The *Mouse 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	<i>(Avoid freeze/thaw cycles!)</i>
71240	Mouse IDO2 His-Tag	400 μ g	-80°C	
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 mouse 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.

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 **IDO2 Reaction Solution** and aliquot 180 μ l into each well.
- 2) Add 10 μ 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 μ l of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.
- 3) Thaw **Mouse IDO2 His-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **Mouse IDO2 His-Tag** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **Mouse IDO2 His-Tag** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 4) Dilute **Mouse IDO2 His-Tag** in **1x IDO2 Assay Buffer** at 400 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

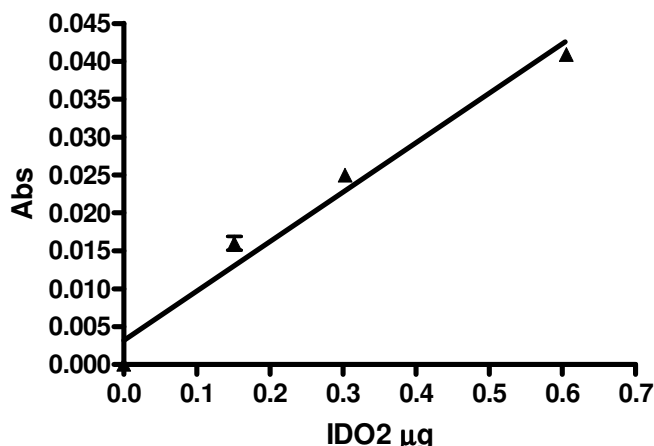
	Blank	Positive Control	Test Inhibitor
IDO2 Reaction Solution	180 μ l	180 μ l	180 μ l
Test Inhibitor	–	–	10 μ l
Inhibitor buffer (no inhibitor)	10 μ l	10 μ l	–
1x IDO2 Buffer	10 μ l	–	–
IDO2 (400 ng/ μ l)	–	10 μ l	10 μ l
Total	200 μl	200 μl	200 μl

- 5) Add 10 μ l of **1x IDO2 Assay Buffer** to the well designated "Blank".
- 6) Initiate reaction by adding 10 μ l of diluted **Mouse 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:



Mouse IDO2 activity measured using the Mouse IDO2 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72042. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

<u>Product</u>	<u>Catalog #</u>	<u>Size</u>
Mouse IDO1, His-tag	71196	50 μg
Mouse IDO2, His-tag	71240	200 μg
Human IDO1, His-tag	71182	50 μg
Human IDO2, His-tag	71194	200 μg
Human TDO, His-tag	71195	50 μg
Human IDO1 Inhibitor Screening Assay Kit	72021	96 rxns
Mouse IDO1 Inhibitor Screening Assay Kit	72041	96 rxns
Human IDO2 Inhibitor Screening Assay Kit	72022	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
hIDO1-HEK293 Recombinant Cell line	60532	2 vials
hTDO-HEK293 Recombinant Cell line	60534	2 vials
NLG919	27337-1	10 mg
NLG919	27337-2	50 mg
INCB024360	27338-1	10 mg
INCB024360	27338-2	100 mg

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