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Data Sheet

Mouse IDO2 Inhibitor Screening Assay Kit

Catalog # 72042

DESCRIPTION: The *Mouse 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	
71240	Mouse IDO2 His-Tag*	2 x 500 μ g	-80°C	(Avoid freeze/thaw cycles!)
	IDO2 Reaction Solution component 1	2 x 10 ml	-80°C	
	IDO2 Reaction Solution component 2	2 x 100 μ l	-80°C	
	IDO2 Substrate	2 x 1 ml	-80°C	
	1x IDO2 Assay Buffer	5 ml	-80°C	
	UV transparent 96-well plate	1	Room Temp.	

*The initial concentration of Mouse IDO2 is lot-specific and will be indicated on the tube containing the enzyme.

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.

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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 component 1** and **component 2**. Prepare **complete IDO2 reaction solution** by mixing 50 μ l of **component 2** with 4.95 ml of **component 1**. Only prepare enough complete reaction solution needed for assay. *Protect from light, and do not re-use freeze-thawed complete IDO2 reaction solution.*
- 2) Add 50 μ l of **complete IDO2 reaction solution** to each well.
- 3) Add 5 μ l of inhibitor solution (containing no more than 10% DMSO in 1x IDO2 assay buffer) to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 5 μ l of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.
- 4) 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.
- 5) Dilute **Mouse IDO2 His-Tag** in **1x IDO2 Assay Buffer** to 400 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
Complete IDO2 Reaction Solution	50 μ l	50 μ l	50 μ l
Test Inhibitor	–	–	5 μ l
Inhibitor buffer (no inhibitor)	5 μ l	5 μ l	–
1x IDO2 Buffer	25 μ l	–	–
Mouse IDO2 (400 ng/ μ l)	–	25 μ l	25 μ l
IDO2 Substrate	20 μ l	20 μ l	20 μ l
Total	100 μl	100 μl	100 μl

- 6) Add 25 μ l of **1x IDO2 Assay Buffer** to the well designated "Blank".

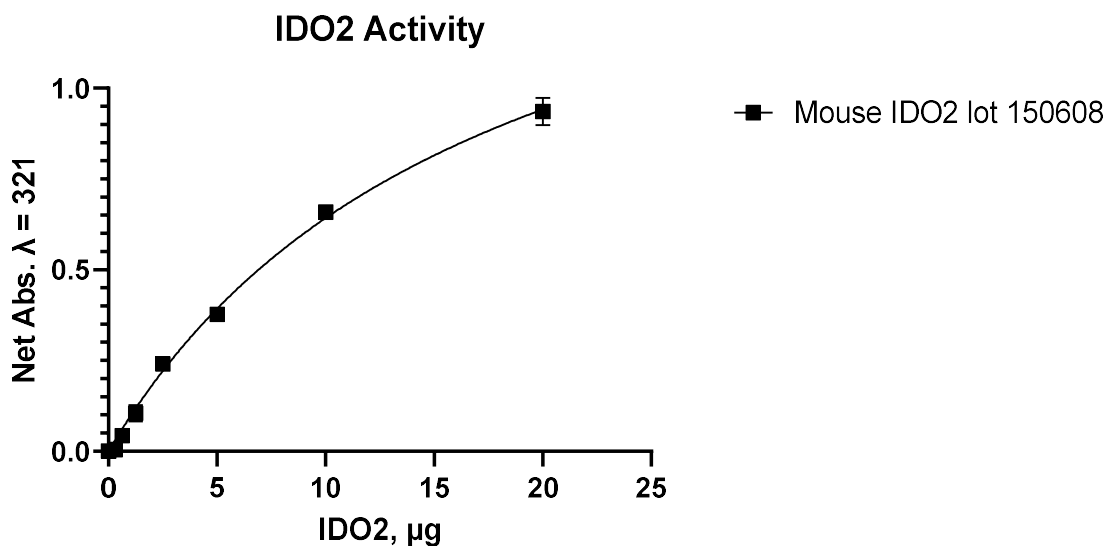
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- 7) Add 25 μ l of diluted **Mouse IDO2 His-Tag** prepared as described above to the wells labeled "Positive Control," and "Test Inhibitor." Cover plate with foil and pre-incubate for 30 minutes at room temperature with slow shaking.
- 8) Initiate reaction by adding 20 μ l of **IDO2 substrate**. Cover plate with foil and incubate for 2 hours at 30 °C.
- 9) Measure absorption at $\lambda=320 - 325$ nm.

EXAMPLE OF ASSAY RESULTS:

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

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RELATED PRODUCTS:

<u>Product</u>	<u>Catalog #</u>	<u>Size</u>
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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