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SZABO-SCANDIC Handels GmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Data Sheet

Mouse IDO1 Inhibitor Screening Assay Kit

Catalog # 72041

DESCRIPTION: The *Mouse IDO1 Inhibitor Screening Assay Kit* is designed to measure inhibition of the murine IDO1 enzyme. The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 100 reactions. This 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	
71196	Mouse IDO1 His-Tag	40 μ g	-80°C	(Avoid freeze/ thaw cycles!)
73001	IDO Reaction Solution	2 x 10 mL	-80°C	
73002	IDO1 Assay Buffer	1 mL	-80°C	
	UV transparent 96-well plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

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

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

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

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

REFERENCES:

1. Koblisch, H.K., *et al.*, Mol Cancer Ther, 2010; **9(2)**: 489-98
2. Liu, X., *et al.*, Blood. 2010; **115(17)**: 3520-3530.
3. 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 **IDO Reaction Solution** and aliquot 180 μ 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 μ 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 μ l of the same solution without inhibitor (inhibitor buffer). *Note: Keep the DMSO concentration below 0.5%.*
- 3) Thaw **IDO1 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **IDO1 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **IDO1 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 4) Dilute **IDO1** in **IDO1 Assay Buffer** at 40 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

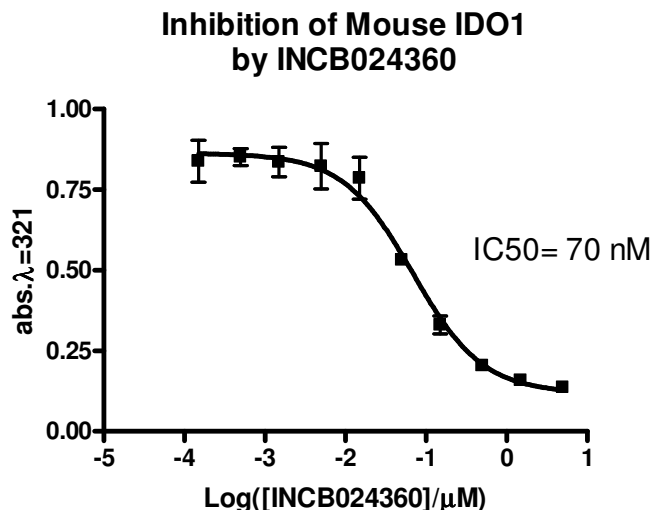
	Blank	Positive Control	Test Inhibitor
IDO1 Reaction Solution	180 μ l	180 μ l	180 μ l
Test Inhibitor	–	–	10 μ l
Inhibitor buffer (no inhibitor)	10 μ l	10 μ l	–
IDO1 Assay Buffer	10 μ l	–	–
Mouse IDO1 (40 ng/ μ l)	–	10 μ l	10 μ l
Total	200 μl	200 μl	200 μl

- 5) Add 10 μ l of **IDO1 Assay Buffer** to the well designated "Blank".
- 6) Initiate reaction by adding 10 μ l of diluted **IDO1** 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:



Inhibition of mouse IDO1 by the inhibitor INCB024360 (Catalog #27338), measured using the mouse IDO1 Inhibitor Screening Assay Kit, Catalog #72041. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog#</u>	<u>Size</u>
Mouse IDO1, His-tag	71196	50 μg
Human IDO1, His-tag	71182	50 μg
Human IDO2, His-tag	71194	50 μg
Human TDO, His-tag	71195	50 μg
PD-1:PD-L1[Biotinylated] Inhibitor Screening Colorimetric Kit	72016	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Colorimetric Kit	72017	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Colorimetric Kit	72019	96 rxns
PD-1:PD-L1[Biotinylated] Inhibitor Screening Chemiluminescent Kit	72003	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Chemiluminescent Kit	72004	96 rxns
PD-1[Biotinylated]:PD-L1 Inhibitor Screening Chemiluminescent Kit	72005	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Chemiluminescent 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
INCB024360	27338-1	10 mg

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