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Data Sheet

IDO1 Inhibitor Screening Assay Kit (384)

Catalog # 72034

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 400 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 $\lambda = 320\text{--}325$ 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	80 μ g	-80°C	(Avoid freeze/thaw cycles!)
73001	IDO1 Reaction Solution	4 x 10 ml	-80°C	
73002	IDO1 Assay Buffer	2 x 1 ml	-80°C	
	UV transparent 384-well plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

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

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

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

STABILITY: Stable at least 6 months from date of receipt, when stored as directed. Kit components require different storage conditions. Be sure to store each component at the proper temperature upon arrival.

REFERENCES:

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 90 μ l into each well. *Note: **IDO1 Reaction Solution** may contain a precipitate after thawing. Please ensure the mixture is fully solubilized by gentle mixing before aliquoting. Do not vortex.*
- 2) Add 5 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 5 μ l of the same solution without inhibitor (inhibitor buffer). *Note: Keep the final 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 Buffer** at 40 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

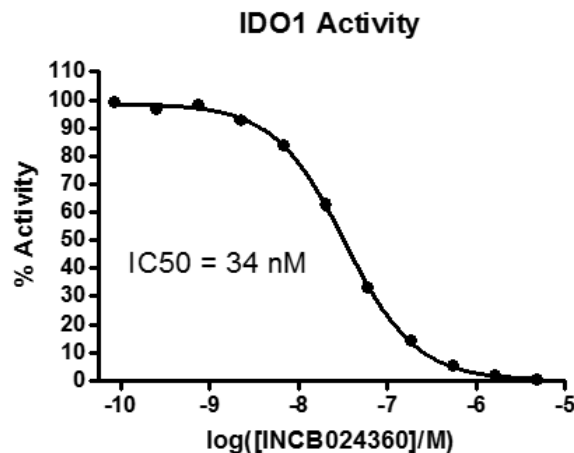
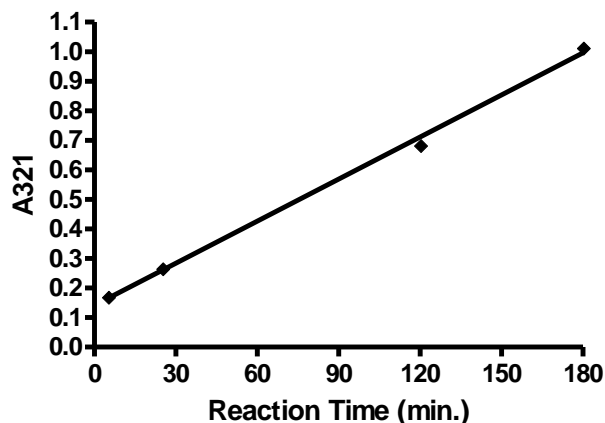
	Blank	Positive Control	Test Inhibitor
IDO1 Reaction Solution	90 μ l	90 μ l	90 μ l
Test Inhibitor	-	-	5 μ l
Inhibitor buffer (no inhibitor)	5 μ l	5 μ l	-
IDO1 Assay Buffer	5 μ l	-	-
IDO1 (40 ng/ μ l)	-	5 μ l	5 μ l
Total	100 μl	100 μl	100 μl

- 5) Add 5 μ l of **IDO1 Assay Buffer** to the well designated "Blank".
- 6) Initiate reaction by adding 5 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. Subtract "Blank" value from all other values.

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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 #72034. *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>
IDO1, His-tag	71182	50 µg
IDO2, His-tag	71194	50 µg
TDO, His-tag	71195	50 µg
IDO1 Inhibitor Screening Assay Kit	72021	96 rxns
IDO2 Inhibitor Screening Assay Kit	72022	96 rxns
TDO Inhibitor Screening Assay Kit	72023	96 rxns
TDO Inhibitor Screening Assay Kit (384)	72036	384 rxns
IDO1 Fluorogenic Inhibitor Screening Assay Kit	72037	96 rxns
TDO Fluorogenic Inhibitor Screening Assay Kit	72039	96 rxns
IDO1 Cell-Based Assay Kit	72031	100 rxns
TDO Cell-Based Assay Kit	72033	100 rxns
NLG919	27337-1	10 mg
INCB024360	27338-1	10 mg
IDO1 Reaction Solution	73001	10 ml
IDO1 Assay Buffer	73002	1 ml
IDO2 Reaction Solution	73003	10 ml
IDO2 Assay Buffer	73004	1 ml
TDO Reaction Solution	73005	10 ml
TDO Assay Buffer	73006	1 ml

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