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Data Sheet
Universal IDO1/IDO2/TDO Inhibitor Screening Assay Kit
Catalog # 72035

DESCRIPTION: The *Universal IDO1/IDO2/TDO Inhibitor Screening Assay Kit* is designed to measure IDO1, IDO2 and TDO enzyme inhibition. The kit comes in a convenient format with enough reaction solution and enzyme to perform a total of 50 reactions with each enzyme (150 reactions total). 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 λ=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) and Tryptophan 2,3 dioxygenase (TDO) are upregulated in many tumors, providing cancer cells with an avenue for immune evasion.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71182	IDO1 His-Tag	20 µg	-80°C	***Avoid freeze/ thaw cycles!***
73001	IDO1 Reaction Solution	10 ml	-80°C	
73002	1x IDO1 Assay Buffer	1 ml	-80°C	
71195	TDO, His-tag	25 µg	-80°C	
73005	TDO Reaction Solution	10 ml	-80°C	
73006	1x TDO Assay Buffer	1 ml	-80°C	
71194	IDO2 His-Tag	500 µg	-80°C	
	IDO2 Reaction Solution component 1	10 ml	-80°C	
	IDO2 Reaction Solution component 2	100 µl	-80°C	
	IDO2 Substrate	1 ml	-80°C	
	1x IDO2 Assay Buffer	5 ml	-80°C	
79965	UV transparent 96-well plate	2	Room Temp.	

MATERIALS REQUIRED BUT NOT SUPPLIED:
Spectrophotometer capable of measuring absorbance at λ=320 – 325 nm
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for the study of IDO1, IDO2, and TDO enzymology, screening inhibitors, and selectivity profiling.

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

STABILITY: At least one year 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.

Assay Protocols: A separate protocol for each enzyme has been provided below. In all cases all samples and controls should be tested in duplicate. Use slow shaking for all incubations.

IDO1 Assay Protocol:

1) Thaw **IDO1 Reaction Solution** and aliquot 180 µl 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 µl of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 10 µl of the same solution without inhibitor (inhibitor buffer). Note: Keep the DMSO concentration below 0.5%.

3) Thaw **IDO1 His-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **IDO1 His-Tag** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **IDO1 His-Tag** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **IDO1 His-Tag** in **1x IDO1 Assay Buffer** at 40 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
IDO1 Reaction Solution	180 µl	180 µl	180 µl
Test Inhibitor	–	–	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	–
1x IDO1 Assay Buffer	10 µl	–	–
IDO1 (40 ng/µl)	–	10 µl	10 µl
Total	**200 µl**	**200 µl**	**200 µl**

5) Add 10 µl of **1x IDO1 Assay Buffer** to the well designated "Blank."

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6) Initiate reaction by adding 10 µl of diluted **IDO1 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 λ=320 – 325 nm.

IDO2 Assay Protocol:

1) Thaw **IDO2 reaction solution component 1** and **component 2**. Prepare **complete IDO2 reaction solution** by mixing 50 µl 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 µl of **complete IDO2 reaction solution** to each well.

3) Add 5 µl 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 µl of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.

4) 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.*

5) Dilute **IDO2 His-Tag** in **1x IDO2 Assay Buffer** to 400 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

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

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

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7) Add 25 µl of diluted **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 µl of IDO2 substrate. Cover plate with foil and incubate for 2 hours at 30 °C.

9) Measure absorption at λ=320 – 325 nm.

TDO Assay Protocol:

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

Step 1:

1) Thaw **TDO Reaction Solution** and aliquot 180 µl into each well. *Note: **TDO 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 µl of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor." For the "Positive Control" and "Blank," add 10 µl of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.

3) Thaw **TDO, His-tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **TDO, His-tag** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **TDO,** **His-tag** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **TDO, His-tag** in **1x TDO Assay Buffer** at 50 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
Reaction Solution	180 µl	180 µl	180 µl
Test Inhibitor	–	–	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	–
1x TDO Assay Buffer	10 µl	–	–
TDO, His-tag (50 ng/µl)	–	10 µl	10 µl
Total	**200 µl**	**200 µl**	**200 µl**

5) Add 10 µl of **1x TDO Assay Buffer** to the well designated "Blank."

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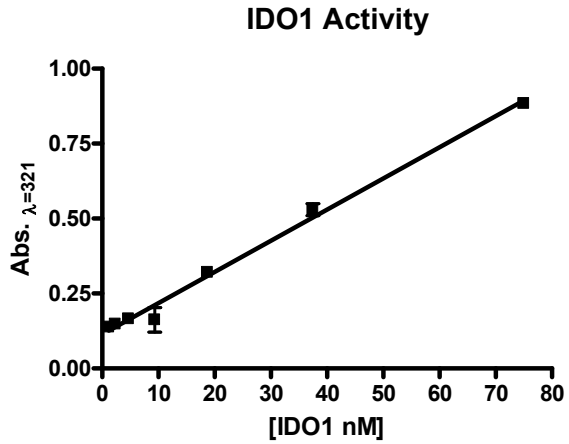
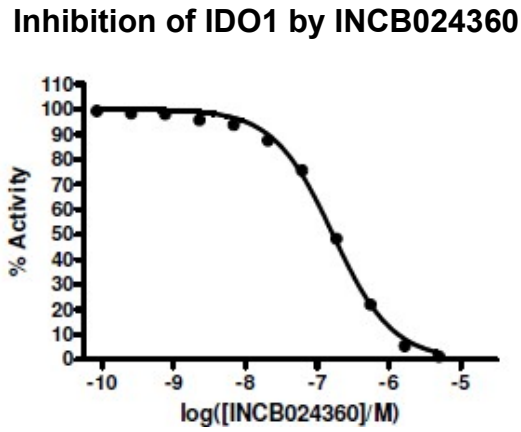
6) Initiate reaction by adding 10 µl of diluted **TDO, His-tag** prepared as described above to the wells labeled "Positive Control," and "Test Inhibitor." Incubate at room temperature for 90 minutes.

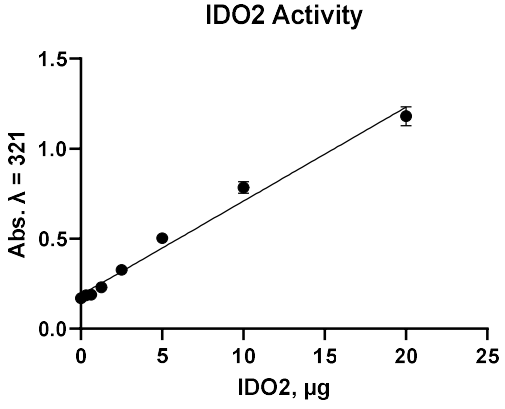
7) Measure absorption at λ=320-325 nm.

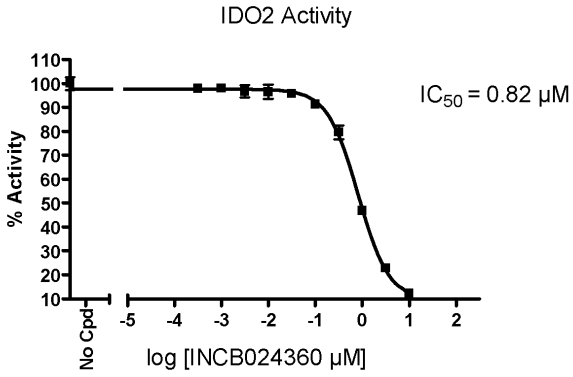
EXAMPLE OF ASSAY RESULTS:





IDO1 activity (right) and IDO1 inhibition (left), measured using the IDO1 protocol for the Universal Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72035. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*





IDO2 activity measured using the IDO2 protocol for the Universal Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72035. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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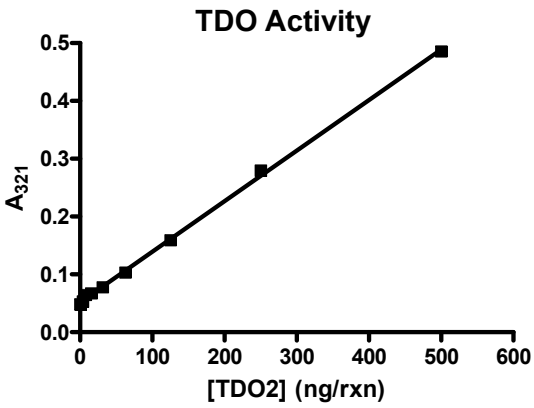

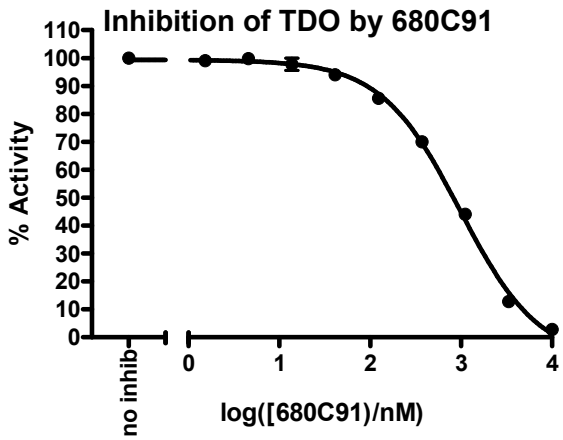

TDO activity (left) and inhibition (right), measured using the TDO protocol for the Universal Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72035. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

Product	Catalog #	Size
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
Human IDO2 Inhibitor Screening Assay Kit	72022	96 rxns
Human TDO Inhibitor Screening Assay Kit	72023	96 rxns
Human IDO1 Cell-Based Assay Kit	72031	100 rxns
Human TDO Cell-Based Assay Kit	72033	100 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
INCB024360	27338-2	100 mg