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Data Sheet

TDO Fluorogenic Inhibitor Screening Assay Kit

Catalog # 72039

DESCRIPTION: The *TDO Fluorogenic Inhibitor Screening Assay Kit* is designed to measure enzyme inhibition of tryptophan 2,3-dioxygenase (TDO). The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 96 reactions. The *TDO Fluorogenic Inhibitor Screening Assay Kit* is simple to use and detects fluorescence at long wavelengths, which minimizes potential errors due to compound interference. In the assay, the inhibitor and enzyme are added to a sample containing L-Trp substrate. After a 1 hour incubation at room temperature, the fluorescence solution is added and incubated at 37°C for four hours. Activity is measured by reading sample fluorescence at $\lambda=510$ nm following excitation of the reaction product at $\lambda=400$ 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., Tryptophan 2,3 dioxygenase (TDO) a rate limiting enzymes in this pathway, is upregulated in many tumors, providing cancer cells with an avenue for immune evasion.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71195	TDO His-Tag	50 μ g	-80°C	(Avoid freeze/ thaw cycles!)
73010	TDO Fluorogenic Reaction Solution	2 x 10 ml	-80°C	
73006	TDO Buffer	1 ml	-80°C	
	Fluorescence Solution	2 x 1 ml	-80°C	
	Black 96 Well Assay-Plate	1		
	Plate sealing film	1		

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorimeter capable of excitation at 390-410 nm and detection at 500-520 nm
Adjustable micropipettor and sterile tips
Rotating or rocker platform

APPLICATIONS: Useful for the study of TDO enzymology, inhibitor screening, and selectivity profiling.

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

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CONTRAINDICATIONS: DMSO >0.5%, strong acids or bases, ionic detergents, high salt.
Warning: the Fluorescence Solution contains a component that is known to be a skin and eye irritant, use caution and appropriate personal protective equipment when handling this component.

REFERENCE: 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 reaction solution and aliquot 180 μ l into each well. Note: TDO 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 10 μ l of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor". For the wells designated "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) Dilute **TDO** in **TDO Buffer** at 50 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
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	-
TDO Buffer	10 μ l	-	-
TDO (50 ng/ μ l)	-	10 μ l	10 μ l
Total	200 μl	200 μl	200 μl

- 4) Add 10 μ l of **TDO Assay Buffer** to the wells designated "Blank".
- 5) Initiate reaction by adding 10 μ l of diluted **TDO** prepared as described above to the wells labeled "Positive Control", and "Test Inhibitor". Incubate at room temperature for 1 hour.
- 6) Add 20 μ l **Fluorescence Solution** to each well. Seal the plate and incubate at 37°C for four hours. Allow plate to cool for 10 min.

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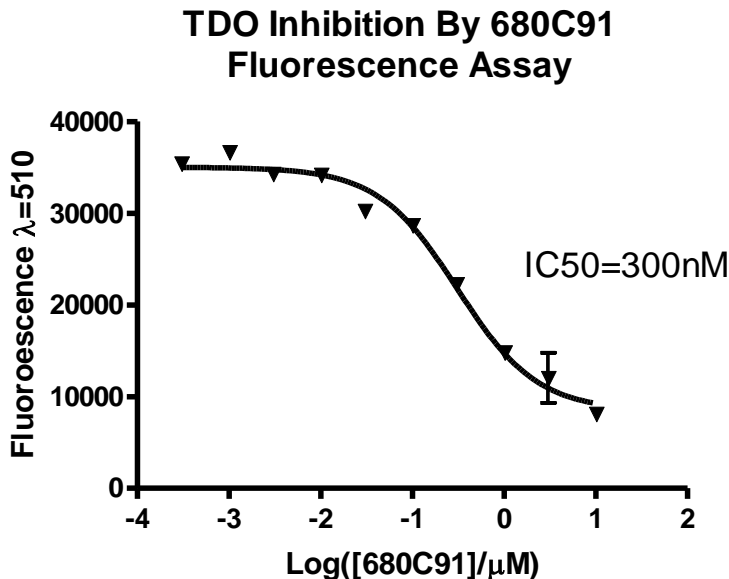
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- 7) Unseal the plate and measure fluorescence in a fluorimeter capable of excitation at 400 nm and emission at 510 nm. Subtract "Blank" value from all other values.

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EXAMPLE OF ASSAY RESULTS:



Inhibition of TDO activity by 680C91, measured using the *TDO Fluorogenic Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog #72039. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS:

Product Name	Catalog #	Size
IDO1, His-tag	71182	50 μg
IDO2, His-tag	71194	50 μg
TDO, His-tag	71195	50 μg
IDO1 Fluorogenic Inhibitor Screening Kit	72034	96 rxns
IDO1 Inhibitor Screening Assay Kit	72021	96 rxns
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
N-formylkynurenine	73000	2 mg
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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