6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

TDO Fluorogenic Inhibitor Screening Assay Kit (384 Well Format)

Catalog # 72049

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 400 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 λ=510 nm following excitation of the reaction product at λ=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*	100 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
73010	TDO Fluorogenic Reaction Solution	3 x 10 ml	-80°C	
73006	TDO Buffer	3 ml	-80°C	
	Fluorescence Solution	4 x 1 ml	-80°C	
79961	Black 384 Well Assay-Plate	1		
	Plate sealing film	1		

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

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.

6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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

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 72 µl 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 4 µl of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor". For the wells designated "Positive Control" and "Blank", add 4 µl 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/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
Reaction Solution	72 µl	72 µl	72 µl
Test Inhibitor	–	–	4 µl
Inhibitor buffer (no inhibitor)	4 µl	4 µl	–
TDO Buffer	4 µl	–	–
TDO (50 ng/µl)	–	4 µl	4 µl
Total	**80 µl**	**80 µl**	**80 µl**

4) Add 4 µl of **TDO Assay Buffer** to the wells designated "Blank".

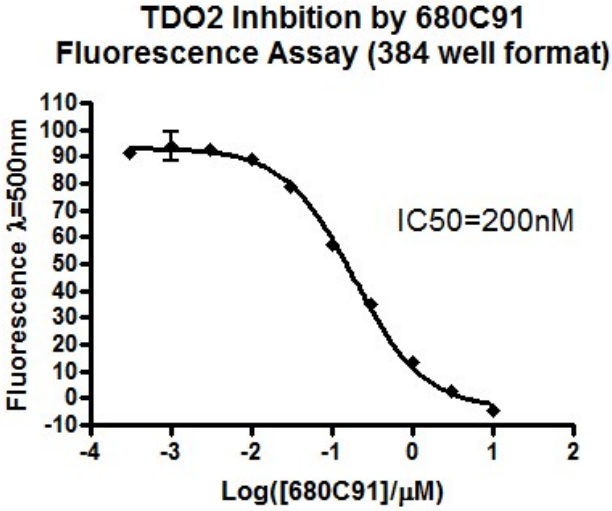
5) Initiate reaction by adding 4 µl of diluted **TDO** prepared as described above to the wells labeled "Positive Control", and "Test Inhibitor". Incubate at room temperature for 1 hour.

6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

6) Add 8 µl **Fluorescence Solution** to each well. Seal the plate and incubate at 37°C for four hours. Allow plate to cool for 10 minutes.

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.

EXAMPLE OF ASSAY RESULTS:



Inhibition of TDO activity by 680C91, measured using the *TDO Fluorogenic Inhibitor Screening Assay Kit (384 well format)*, BPS Bioscience, Catalog # 72049. *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
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
TDO-HEK293 Recombinant Cell line	60534	2 vials
TDO Cellular Activity QuickDetect™ Supplements	62002-1	100 rxns
N-formylkynurenine	73000	2 mg
NLG919	27337-1	10 mg
INCB024360	27338-1	10 mg
TDO Reaction Solution	73005	10 ml
TDO Assay Buffer	73006	1 ml