Description

The TDO2 Fluorogenic Inhibitor Screening Assay Kit – 384 (Human) is designed to measure TDO2 (tryptophan 2,3-dioxygenase 2) activity for screening and profiling applications. The assay kit comes in a convenient 384-well format, with enough recombinant TDO2, fluorogenic substrate and solution, and assay buffer for 400 enzyme reactions.

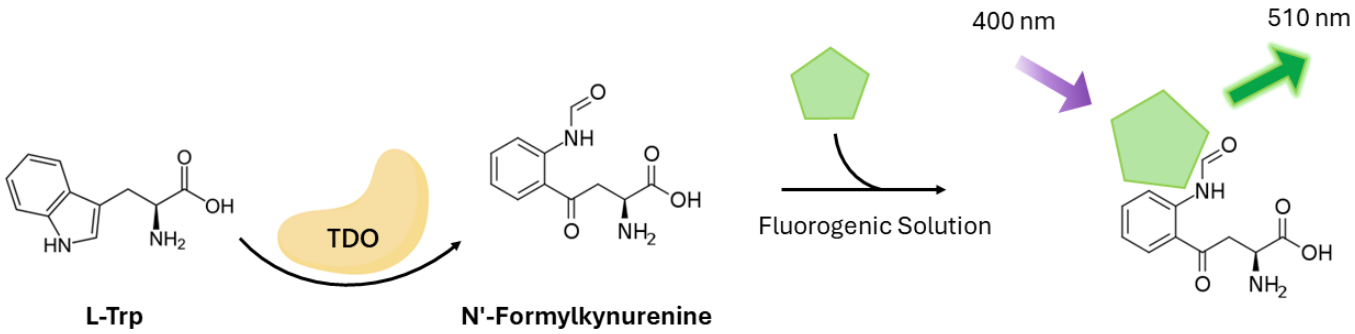

Figure 1: Illustration of the mechanism behind the TDO2 Fluorogenic Inhibitor Screening Assay Kit – 384 (Human). TDO2 converts the conversion of tryptophan to N-formylkynurenine (NFK), which can react with the TDO Fluorogenic reagent present in the TDO Fluorogenic Reaction Solution and generate a green fluorescent molecule. The increase in fluorescence is proportional to TDO2 activity.

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. TDO (Tryptophan 2,3 dioxygenase) is a heme-containing protein, involved in the catabolism of L-Trp to Kyn. Its activity results in the consumption of L-Trp and an accumulation of Kyn. This metabolism maintains an immunosuppressive microenvironment by starving immune cells of L-Trp and releasing degradation products of L-Trp that have immunosuppressive functions. Accumulation of Kyn also has an impact on neurons and brain related diseases. TDO is upregulated in many tumors, providing cancer cells with an avenue for immune evasion. The use of TDO inhibitors may be a viable therapeutic strategy in cancer therapy and neuroscience related disorders.

Applications

Study enzyme kinetics and screen small molecule inhibitors of TDO for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71195	TDO2, His-Tag (Human)*	100 µg	-80°C
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, low binding 384- microtiter plate	1	Room Temperature
	Plate Sealing Film	1	Room Temperature

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

Materials Required but Not Supplied

- Fluorimeter capable of excitation at λ=390-410 nm and detection at λ=500-520 nm
- 37°C incubator
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 0.5%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.
- The presence of strong acids or bases, ionic detergents and high salt should be avoided.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using 680C91 (#82607) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **TDO Fluorogenic Reaction Solution.** Mix well.

   Note: This solution may show the presence of a precipitate after thawing. Make sure the precipitate is dissolved by mixing prior to adding to the wells. Do not vortex.

2. Add 72 µl of TDO Fluorogenic Reaction Solution into each well.

3. Prepare the **Test Inhibitor** (4 µl/well): for a titration, prepare serial dilutions at concentrations 20-fold higher than the desired final concentrations. The final volume of the reaction is 80 µl.

   3.1 If the Test Inhibitor is water-soluble, prepare 20-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in water.

   For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   3.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 200-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 20-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using distilled water containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 20-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 0.5%.

4. Add 4 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

5. Add 4 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

6. Thaw TDO Buffer.

7. Thaw TDO2 on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

8. Dilute TDO2 to 50 ng/µl with TDO Buffer (4 µl/well).

9. Initiate the reaction by adding 4 µl of diluted TDO2 to the "Positive Control" and "Test Inhibitor" wells.

10. Add 4 µl of TDO Buffer to the "Blank" wells.

11. Incubate at Room Temperature (RT) for 1 hour with gentle agitation.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
TDO Fluorogenic Reaction Solution	72 µl	72 µl	72 µl
Test Inhibitor	-	-	4 µl
Diluent Solution	4 µl	4 µl	-
TDO Buffer	4 µl	-	-
Diluted TDO2 (50 ng/µl)	-	4 µl	4 µl
Total	**80 µl**	**80 µl**	**80 µl**

12. Thaw Fluorescence Solution.

13. Add 8 µl of Fluorescence Solution to each well.

14. Seal the plate and incubate for 4 hours at 37°C with gentle agitation.

15. Cool the plate at RT for 10 minutes.

16. Remove the sealer and read in a fluorimeter capable of excitation at λ=400 nm and detection at λ=510 nm.

    Note: If condensation is observed it is recommended to centrifuge the plate prior to removing the seal. Wells that present significant volume changes should be excluded from data analysis.

17. The "Blank" value is subtracted from all other readings.

Example Results

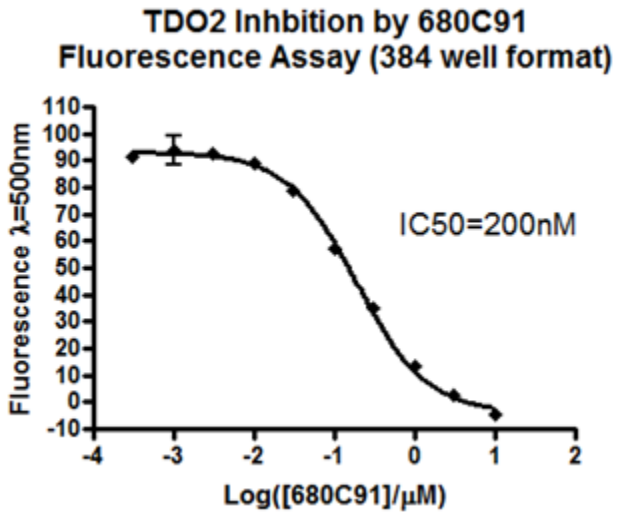

Figure 2: Inhibition of TDO2 activity by the inhibitor 680C91.
TDO2 activity was measured in the presence of increasing concentrations of 680C91. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Seegers, N., *et al.*, 2014 J. *Biomol. Screen.* 19(9):1266-74.
Yu C., *et al.*, 2016 *Metab Brain Dis* 31(4):737-47.

Related Products

Products	*Catalog #*	*Size*
TDO- HEK293 Recombinant Cell Line	60534	2 vials
TDO Cellular Activity QuickDetect™ Supplements	62002	100 reactions/1000 reactions
TDO Inhibitor Screening Assay	72036	384 reactions
TDO Cell-Based Assay Kit	72033	100 reactions
TDO Primer Mix-qPCR Assay	71271	96 reactions

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