6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

IDO1 Inhibitor Mechanism of Action Assay Kit

Catalog #: 72050 Size: 50 reactions

DESCRIPTION: The *IDO1 Inhibitor Mechanism of Action (MoA) Assay Kit* is designed to determine the mechanism of IDO1 enzyme inhibition (e.g., reversible or irreversible inhibition). The kit comes in a convenient format with enough reaction solution and enzyme to perform a total of 50 reactions. The *IDO1 Mechanism of Action Assay Kit* is simple to use. Inhibitor and enzyme are preincubated allowing the inhibitor to bind. After preincubation, the mixture is diluted into reaction buffer containing the L-tryptophan (L-Trp) substrate and all necessary coupled reaction components. This allows reversible inhibitors to dissociate, while irreversible inhibitors remain associated with the IDO1 enzyme. IDO1 activity is determined by measuring the absorption of the reaction product at λ=320 – 325 nm.

BACKGROUND: 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.

MECHANISM OF THE ASSAY: Inhibitors of IDO1 can bind either irreversibly (covalent binding to the protein) or reversibly (non-covalent association) to the enzyme. The *IDO1 Inhibitor Mechanism of Action Assay Kit* allows researchers to determine the mechanism of IDO1 inhibitor binding. At high concentrations, e.g., 10x the IC_{50} concentration of the inhibitor, both reversible and irreversible inhibitors will interfere with IDO1 activity. However, after sufficient dilution, e.g. to 0.3x the IC_{50} concentration, reversible inhibitors will dissociate, relieving inhibition of the IDO1 enzyme. Irreversible inhibitors will not dissociate, and will continue to inhibit the IDO1 enzyme at the same level. Therefore, by monitoring enzymatic activity after inhibitor dilution, the mechanism of binding to the IDO1 protein can be determined.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71182	IDO1 His-Tag	40 µg	-80°C	***Avoid freeze/ thaw cycles!***
73001	IDO1 Reaction Solution	10 ml	-80°C	
73002	1x IDO1 Assay Buffer	25 ml	-80°C	
	Reversible Reference Inhibitor (1000x)	50 µl	-20°C	
	Irreversible Reference Inhibitor (1000x)	50 µl	-20°C	
	UV transparent 96-well Reaction Plate	1	Room Temp.	
	96-well Preincubation Plate	1	Room Temp.	

6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

MATERIALS REQUIRED BUT NOT SUPPLIED:
Spectrophotometer capable of measuring absorbance at λ=320 – 325 nm
Adjustable micropipettor and sterile tips
Sample inhibitors, at 1000x the IC_{50} concentration
Dilution plate to prepare inhibitor dilutions

Note: *Prior to the assay, the approximate IC_{50} of each test inhibitor should be determined. This can be determined using the Human IDO1 Inhibitor Screening Assay Kit, #72021.*

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

CONTRAINDICATIONS:
DMSO > 1%, 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.
3. Strelow, J., *et al.* Assay Guidance Manual. 2012

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

1) Thaw **IDO1 Reaction Solution** and aliquot 184 µl into each well of the UV transparent assay plate. *Note: **IDO1 Reaction Solution** may contain a precipitate after thawing. Please ensure the mixture is fully solubilized by mixing thoroughly before aliquotting. Do not vortex.*

   Aliquot reaction solution into enough wells for two enzyme control conditions ("*Blank"* and "*Positive Control"*), three conditions for each reference inhibitor, and three conditions for each inhibitor to be tested ("*0.3x IC_{50}*," "*10x IC_{50},"* and "*Test condition"*). All conditions should be prepared in duplicate.

   (Note: Total number of wells needed = 16 for controls [2 blank, 2 positive control, 6 reversible reference, and 6 irreversible reference] + 6 x N; where N = number of sample inhibitors.)

2) *Prepare inhibitor dilutions***.** (A plate to prepare these dilutions is not provided as a part of the assay kit) - Each inhibitor to be tested (including **Reversible Reference Inhibitor** and **Irreversible Reference Inhibitor**) should be diluted to two concentrations in 100% DMSO. The highest concentration should be 1000x the IC_{50} and from this dilute 1:30 with DMSO to create a 30x stock.

6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

From these 100% DMSO inhibitor stocks two parallel sets of dilutions should be made, one for the **Assay Plate** and one for the **Preincubation Plate**. In each set of dilutions there should be three conditions for each inhibitor (the "*0.3x IC_{50}*," the "*10x IC_{50}*," and the "*Test condition*." See **Figure 1**.

3) For the **Assay Plate,** dilute the 100% DMSO inhibitor stocks 1:5 into **1x IDO1 Assay buffer** as follows:
   For the two enzyme control conditions (*Blank* and *Positive Control*) dilute 100% DMSO.

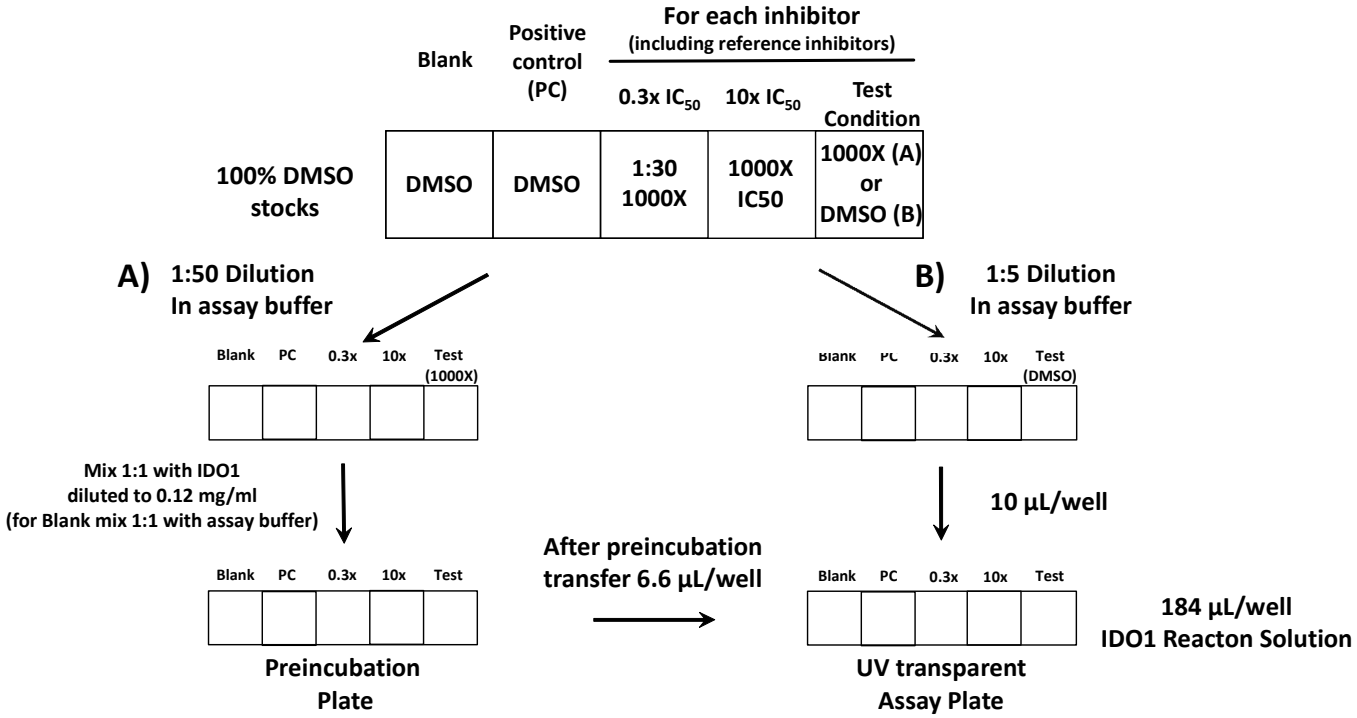
   For the "*0.3x IC_{50}"* dilute the 30x stock for each inhibitor (reference inhibitors and sample inhibitors)

   For the "*10x IC_{50}"* dilute the 1000x stock for each inhibitor (reference inhibitors and sample inhibitors)

   For the "*Test condition"* dilute 100% DMSO

   Add 10 µl of the 1:5 dilutions to the reaction solution in the UV transparent assay plate for each replicate.

Figure 1. Dilution Scheme



6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

4) For the **Preincubation Plate** dilute the 100% DMSO inhibitor stocks 1:50 into **1x IDO1 Assay buffer** as follows:

   For the two enzyme control conditions dilute 100% DMSO.

   For the "*0.3x IC_{50}"* dilute the 30x stock for each inhibitor (reference inhibitors and sample inhibitors)

   For the "*10x IC_{50}"* dilute the 1000x stock for each inhibitor (reference inhibitors and sample inhibitors)

   For the "*Test condition*" dilute 1000x stock for each inhibitor (reference inhibitors and sample inhibitors)

   Add 5 µl of the 1:50 dilutions to wells in the preincubation plate.*

5) 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.*

6) Dilute **IDO1 His-Tag** in **1x IDO1 Assay Buffer** at 0.12 mg/ml. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

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

8) Initiate inhibitor binding by adding 5 µl of diluted **IDO1 His-Tag** prepared as described above to all other wells on the **Preincubation Plate**. Incubate at room temperature for 30 minutes.

9) After 30 minutes transfer 6.6 µl from each preincubation well to wells in the **Assay Plate**. Mix by gently pipetting up and down a few times.

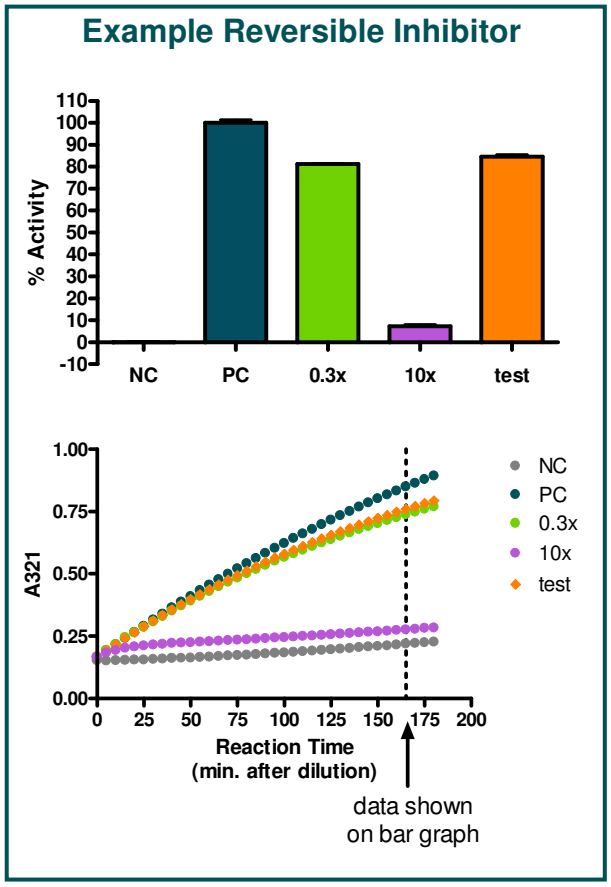
   Incubate at room temperature for up to 180 minutes.

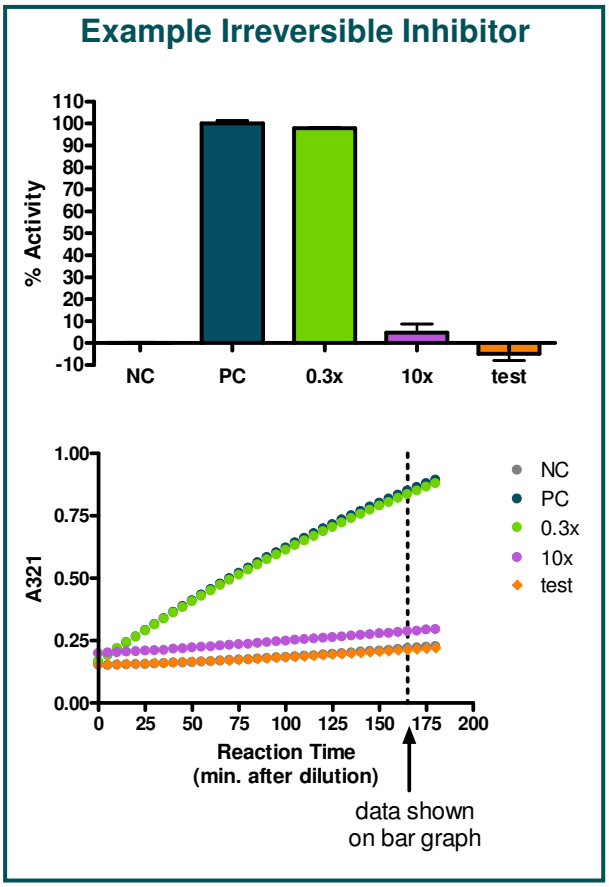
10) Measure absorption at λ=320 – 325 nm.

   *Note: If desired, in steps 4-8 the duplicate wells for each condition can be consolidated into single wells to minimize handling by doubling the volume at each step. Then proceed to step 9 and transfer 6.6 µl from each well into the two duplicate wells in the assay plate.

6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

EXAMPLE OF ASSAY RESULTS:





The compounds were diluted in DMSO and pre-incubated with IDO1 in the absence of substrate, then diluted into reaction solution containing substrate, the coupled reaction components, and the indicated concentrations of inhibitor. The reaction mixture incubated at room temperature reading UV absorption signal every 5 min.

The controls included are the **negative control (NC)**, in which assay buffer was added instead of IDO1, **(PC) no inhibitor** throughout to reflect full enzyme activity at the amount of DMSO tested, **(0.3x) 0.3x IC_{50}** throughout to reflect the expected amount of inhibition remaining after dilution (if fully and rapidly reversible) and **(10x) 10x IC_{50}** throughout to achieve 100% inhibition (and signal expected for irreversible inhibition). The **(test) conditions** were pre-incubated at 10×IC_{50} and then diluted to 0.3×IC_{50}. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*