Description

The Kynurenine 3-Monooxygenase (KMO) Inhibitor Screening Assay Kit is designed to measure human KMO enzyme inhibition. The kit comes in a convenient format, with enough reaction solution and enzyme to perform 384 reactions. The KMO Inhibitor Screening Assay Kit is simple to use. Recombinant human KMO is mixed with the test inhibitor and the reaction is initiated through addition of substrates NADPH and L-Kynurenine. After incubation, KMO activity is determined by measuring the absorption of the reaction product at λ=340 nm. The UV absorption signal correlates with the amount of NADPH remaining in the reaction, therefore the signal is inversely related to the enzymatic activity.

Background

Overexpression of IDO1/2 (indoleamine 2,3-dioxygenase) and TDO (tryptophan 2,3-dioxygenase) in tumor cells promotes tryptophan depletion in the microenvironment, resulting in suppression of the T cell-mediated immune response. IDO1/2 and TDO catalyze the breakdown of tryptophan into kynurenine, and KMO plays a key role in that pathway by hydroxylating kynurenine into 3-hydroxykynurenine. KMO is required for the synthesis of quinolinic acid, a neurotoxic NMDA receptor antagonist involved in axonal targeting, synaptogenesis and apoptosis during brain development. KMO activity has been linked to Huntington's and Alzheimer's diseases, and research shows that KMO inhibitors can improve the lifespan of mice with Huntington's disease. In addition to being a therapeutic target for neurological disorders, elevated KMO is an indicator of renal and hepatocellular carcinoma, suggesting that KMO may have value as a prognostic biomarker.

Applications

Study enzyme kinetics and screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
11307	KMO, His-FLAG Tag*	200 µg	-80°C
	3X KMO Assay Buffer	20 ml	-20°C
	L-Kynurenine (L-Kyn, 20 mM)	500 µl	-80°C
	NADPH (10 mM)	500 µl	-80°C
	UV transparent 384-well plate	1	Room temp.

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

Materials Required but Not Supplied

Name	Catalog #
Spectrophotometer capable of measuring absorption of reaction product in the UV range at λ=300-400 nm	

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.

Assay Principle

UV Absorbance assays quantify the amount of a particular reagent by measuring the amount of light absorbed by the reagent at a specific wavelength in the UV range. This assay relies on UV light absorption by substrate NADPH. The amount of NADPH is proportional to light absorption at λ=340 nm. As the enzyme uses the substrate, less light is absorbed and the signal decreases, therefore the signal in this assay in inversely proportional to enzymatic activity. The "negative control" is performed with substrate but in the absence of enzyme, and corresponds to the highest signal. The "positive control" is performed with the enzyme in the absence of inhibitor, and corresponds to the highest enzymatic activity level of the assay and to the lowest signal.

Contraindications

DMSO > 1%, strong acids or bases, ionic detergents, high salt.

Assay Protocol

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

Preparing Your Reagents

1. Dilute 3X KMO assay buffer to 1X KMO assay buffer with water. Dilute only enough 3X KMO assay buffer required for the assay; store the remaining 3x KMO assay buffer as directed.

2. Thaw KMO enzyme on ice. Briefly spin the tube to recover its full contents.

3. Dilute **KMO enzyme** using **1X KMO Assay Buffer** to a concentration of 20 µg/ml (you will need 25 µl/well). Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

   Notes: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

   Calculate the amount of protein required and dilute enough for your assay. Aliquot unused protein into 2-4 aliquots as may be desired (single use aliquots) and store them at -80°C. *Avoid multiple freeze/thaw cycles. Do not re-use the aliquots more than once and do not re-use the diluted protein.*

4. Add 25 µl of 1X KMO Assay Buffer to the well designated "Blank".

5. Add 25 µl of diluted KMO (20 µg/ml) to the wells designated "Positive Control," and "Test Inhibitor."

6. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%. Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

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

7. Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the wells labeled "Positive Control" and "Blank", add 5 µl of the diluent solution without inhibitor as explained above.

   Keep DMSO concentration of the Test Inhibitor at ≤10% as final DMSO concentration in the reaction should be ≤1%.

Initiating The Reaction

8. Prepare the Substrate Mixture by adding 400 µl of NADPH (10 mM) and 400 µl of L-Kyn (20 mM) to 8.2 ml of 1X KMO Assay Buffer.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
KMO (20 µg/ml)	-	25 µl	25 µl
1X KMO Assay Buffer	25 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Substrate Mixture	20 µl	20 µl	20 µl
Total	50 µl	50 µl	50 µl

9. Initiate the reaction by adding 20 µl of Substrate Mixture, prepared as described above, to all wells.

10. **Measure the absorption at λ=340 nm at time 0. This is an important step as it will account for the possible absorption of the test compound at λ=340 nm.**

11. Incubate at room temperature for 90 minutes.

    At the end of the incubation period, measure absorption at λ=340 nm.

Data Analysis

This absorbance assay measures a signal that correlates with the amount of NADPH remaining in the reaction and is inversely related to the activity. Reading is performed at time 0 as well as 90 minutes. For all conditions, the final timepoint at 90 minutes is subtracted from the initial timepoint at 0 min (net activity). Net activity in the absence of test inhibitor is used as positive control and set to 100%. The negative control provides the 0% of the assay in the absence of inhibitor. The percent activity for each test compound concentration is therefore calculated using this equation:

$$\% \text{ activity} = \frac{(time\ 0\ value - time\ 90'\ value)x100}{positive\ control\ value}$$

Example Results

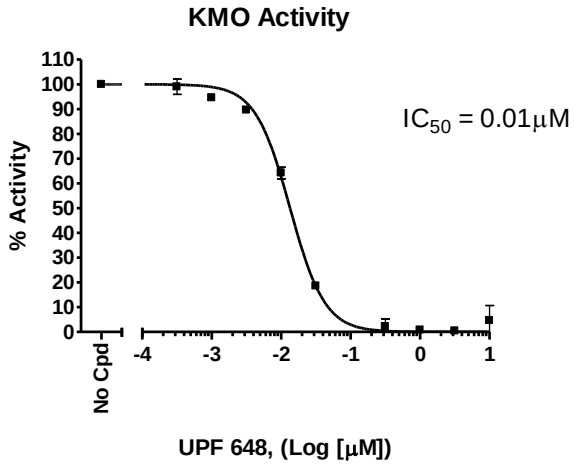

Figure 1: KMO activity measured using the KMO Inhibitor Screening Assay Kit (BPS Bioscience #79513-2). KMO enzymatic activity was measured in the presence of increasing concentrations of inhibitor UPF648. Data was analyzed as described above and are expressed as % of Net activity, with the positive control condition set as 100%.

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

1. Schwarcz, R., and Stone, T.W. 2017. *Neuropharmacology*. Jan; **112(Pt B)**: 237–247.
2. Jin, H., et al. 2015. *Sci Rep*. **5**: 10466.

Related Products

Products	*Catalog #*	*Size*
KMO, His-FLAG-Tags	11307	100 µg
KYNU, His-Tag	79485	20 µg
N-formylkynurenine	73000	2 mg
IDO1, His-Tag	71182	50 µg/500 µg
IDO2, His-Tag	71194	100 µg
TDO, His-Tag (Human)	71195	50 µg
IDO1 Inhibitor Screening Assay Kit	72021	96 reactions
IDO2 Inhibitor Screening Assay Kit	72022	96 reactions
TDO Inhibitor Screening Assay Kit	72023	96 reactions
IDO1 Cell-Based Assay Kit	72031	100 reactions
TDO Cell-Based Assay Kit	72033	100 reactions
IDO1-HEK293 Recombinant Cell line (Human)	60532	2 vials
TDO-HEK293 Recombinant Cell line	60534	2 vials
IDO1 Cellular Activity QuickDetect™ Supplements	62000	100 reactions
NLG919	27337-1	10 mg/50 mg
INCB024360 Analog	27338	10 mg/100 mg