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Data Sheet

KMO Inhibitor Screening Assay Kit (96-well)

Catalog # 79513-1

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 a total of 96 reactions. The *KMO Inhibitor Screening Assay Kit* is simple to use. Recombinant hKMO is mixed with inhibitors and the reaction is initiated through addition of the substrates NADPH and L-Kynurenine. After a room temperature incubation, 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.

BACKGROUND: IDO1/2 and TDO overexpression 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, 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 Huntington's disease mice. In addition to being a therapeutic target for neurological disorders, elevated KMO also is an indicator of renal and hepatocellular carcinoma, suggesting KMO may have value as a prognostic biomarker.

COMPONENTS:

Catalog #	Component	Amount	Storage	
11307	KMO, His-FLAG Tag	100 µg	-80°C	***Avoid freeze/thaw cycles***
	3X KMO Assay Buffer	10 ml	-20°C	
	L-Kynurenine (L-Kyn, 20mM)	500 µl	-80°C	
	NADPH (10 mM)	500 µl	-80°C	
	UV transparent 96-well half area plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Spectrophotometer capable of measuring absorption of reaction product at λ =300-400 nm

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

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

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STABILITY: Stable at least 6 months from date of receipt, when stored as directed. Kit components require different storage conditions. Be sure to store each component at the proper temperature upon arrival.

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.

ASSAY PROTOCOL:

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

Step 1:

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 remaining 3x buffer as directed.

2) Thaw **KMO enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **KMO enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **KMO enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute **KMO** with **1X KMO Assay Buffer** to 20 µg/ml. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
KMO (20 µg/ml)	–	50 µl	50 µl
1X KMO Assay Buffer	50 µl	–	–
Test Inhibitor	–	–	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	–
Substrate Mixture	40 µl	40 µl	40 µl
Total	**100 µl**	**100 µl**	**100 µl**

4) Add 50 µl of **1X KMO Assay Buffer** to the well designated “Blank”.

5) Add 50 µl of **KMO (20 µg/ml)** to the wells designated “Positive Control,” and “Test Inhibitor.”

6) Add 10 µl of inhibitor solution (containing not more than 10% DMSO) to each well designated “Test Inhibitor”. For the wells labeled “Positive Control” and “Blank”, add 10 µl of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 1%.

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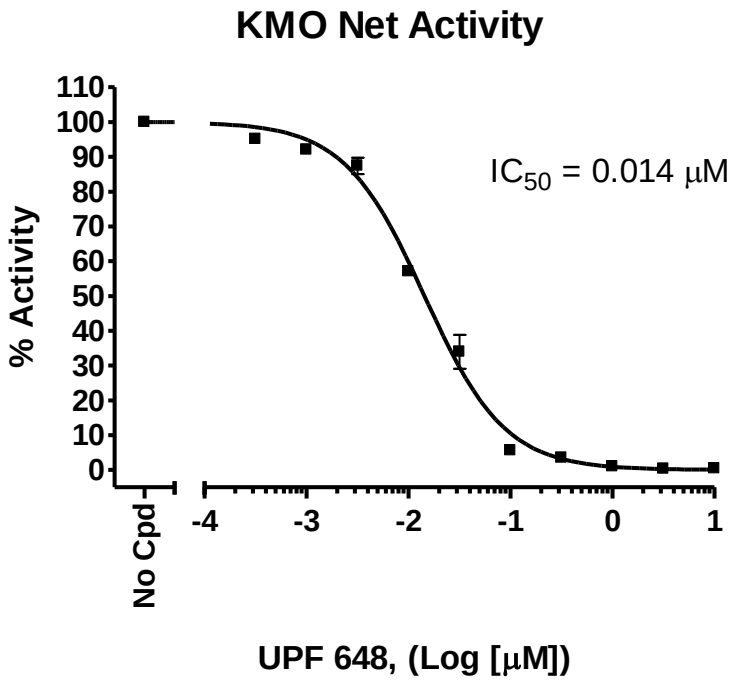
7) Prepare **Substrate Mixture** by adding 400 µl **NADPH (10 mM)** and 400 µl **L-Kyn (20mM)** to 8.2 ml **1X KMO Assay Buffer**.

8) Initiate reaction by adding 40 µl of **Substrate Mixture**, prepared as described above, to all wells. Incubate at room temperature for 1.5 hours.

9) Measure absorption of reaction product at λ =340 nm. *It is recommended to read the plate at time 0 as well as the final timepoint at 90 min. The time 0 measurement can be subtracted from the final reading to account for compound absorbance (net activity).*

EXAMPLE OF ASSAY RESULTS:



KMO inhibition measured using the KMO Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #79513-1. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

Product Name	Catalog#	Size
KMO Inhibitor Screening Assay Kit	79513	384 rxns
KMO, His-FLAG-Tags	11307	50 µg
KYNU, His-Tag	79485	50 µg
N-formylkynurenine	73000	2 mg
IDO1, His-tag	71182	50 µg
IDO2, His-tag	71194	50 µg
TDO, His-tag	71195	50 µg
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
IDO1-HEK293 Recombinant Cell line	60532	2 vials
TDO-HEK293 Recombinant Cell line	60534	2 vials
IDO1 Cellular Activity QuickDetect™ Supplements	62000-1	100 rxns
NLG919	27337-1	10 mg
INCB024360	27338-1	10 mg