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Data Sheet

Kynureninase Inhibitor Screening Assay Kit

Catalog #79514

DESCRIPTION: The *Kynureninase (KYNU) Inhibitor Screening Assay Kit* is designed to measure human KYNU enzyme inhibition. The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 100 reactions. The *KYNU Inhibitor Screening Assay Kit* is simple to use. Inhibitor and enzyme are mixed and the reaction is initiated through addition of the substrate 3-hydroxy-DL-kynurenine (3-HK). After a room temperature incubation, activity is determined by measuring the fluorescence of the reaction product (ex. 315 nm, em. 415 nm).

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 subsequently KYNU cleaves kynurenine into anthranilic acid, a precursor of tryptophan. High levels of KYNU have been found in lymphoma, breast, liver, pancreas, and urothelial cancers, and is thought to promote tumor aggressiveness and metastasis. KYNU levels are also elevated in many chronic inflammatory skin diseases such as psoriasis and atopic dermatitis inflammatory, making KYNU attractive as a potential therapeutic and prognostic target.

COMPONENTS:

Catalog #	Component	Amount	Storage	
79485	KYNU His-Tag	2 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	3X KYNU Assay Buffer	10 ml	-20°C	
	10X 3-HK Substrate	0.5 ml	-80°C	
	Black, low binding NUNC microtiter plate	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Spectrophotometer capable of measuring fluorescence at excitation of 310-320 nm, and emission of 410-420 nm.

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

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

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.

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REFERENCES:

1. Phillips, R.S. 2014. *Arch Biochem Biophys*. Feb 15; **544:**69-74.
2. Badawy, AA. 2017. *Int J Tryptophan Res.* Mar 15;10: 1178646917691938.
3. Harden, J.L., *et al.* 2016. *J Allergy Clin Immunol*. Jun;**137(6):**1830-1840.

ASSAY PROTOCOL:

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

Step 1:

1) Dilute **3X KYNU assay buffer** to **1X KYNU assay buffer** with water**.**

2) Dilute **10X 3-HK substrate** with 1X KYNU assay buffer to make 1X 3-HK substrate.

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

4) Dilute **KYNU** with **1X KYNU Assay Buffer** to 0.2 µg/ml. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
KYNU (0.2 µg/ml)	–	50 µl	50 µl
1X KYNU Assay Buffer	50 µl	–	–
Test Inhibitor	–	–	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	–
1X 3-HK substrate	40 µl	40 µl	40 µl
Total	**100 µl**	**100 µl**	**100 µl**

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

6) Add 50 µl of **KYNU (0.2 µg/ml)** to the wells designated “Positive Control,” and “Test Inhibitor.”

7) Add 10 µl of inhibitor solution (containing no 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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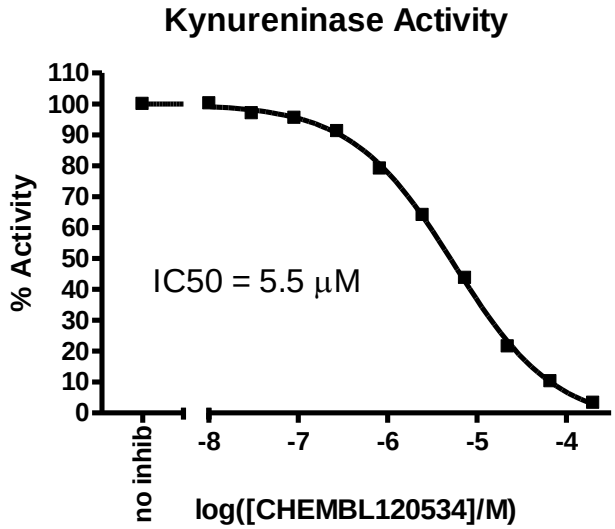
8) Initiate reaction by adding 40 µl of 1X 3-HK substrate, prepared as described above, to all wells. Incubate at room temperature for 15 minutes.

9) After incubation measure fluorescence (ex. 315 nm, em 415 nm).
If compounds are fluorescent, it is recommended to read the plate at time 0 as well as the final timepoint at 15 min. The time 0 measurement can be subtracted from the final reading to account for compound fluorescence.

EXAMPLE OF ASSAY RESULTS:



KYNU inhibition measured using the KYNU Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #79514. *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
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
N-formylkynurenine	73000	2 mg
NLG919	27337-1	10 mg
INCB024360	27338-1	10 mg

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