Data Sheet

IDO1 Fluorogenic Inhibitor Screening Assay Kit (384 Well Format)

Catalog # 72047

DESCRIPTION: The *IDO1 Fluorogenic Inhibitor Screening Assay Kit* is designed to measure enzyme inhibition of indoleamine 2,3-dioxygenase 1 (IDO1). The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 400 reactions. The *IDO1 Fluorogenic Inhibitor Screening Assay Kit* is simple to use and detects fluorescence at long wavelengths, which minimizes potential errors due to compound interference. In the assay, the inhibitor and enzyme are added to a sample containing L-Trp substrate. After a 1 hour incubation at room temperature, the fluorescence solution is added and incubated at 37ºC for four hours. Activity is measured by reading sample fluorescence at λ=510 nm following excitation of the reaction product at λ=400 nm.

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. 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.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71182	IDO1 His-Tag	2 x 105 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
73009	IDO1 Fluorogenic Reaction Solution	5 x 10 ml	-80°C	
73002	IDO1 Buffer	5 ml	-80°C	
	Fluorescence Solution	6 ml	-80ºC	
	Black 384 Well Assay-Plate	1		
	Plate sealing film	1		

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorimeter capable of excitation at 390-410 nm and detection at 500-520 nm
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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

STABILITY: At least one year from date of receipt when stored as directed.

CONTRAINDICATIONS: DMSO >0.5%, strong acids or bases, ionic detergents, high salt. *Warning: the Fluorescence Solution contains a component that is known to be a skin and eye irritant. Use caution and appropriate personal protective equipment when handling this component.*

REFERENCE(S):
1. Seegers, N., *et al. J. Biomol. Screen*. 2014. **19(9):**1266-74.

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

Step 1:
1) Thaw reaction solution and aliquot 108 µl into each well.

2) Add 6 µl of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor." For the wells designated "Positive Control" and "Blank," add 6 µl of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.

3) Dilute **IDO1** in **IDO1 Buffer** at 80 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
Reaction Solution	108 µl	108 µl	108 µl
Test Inhibitor	–	–	6 µl
Inhibitor buffer (no inhibitor)	6 µl	6 µl	–
IDO1 Buffer	6 µl	–	–
IDO1 (80 ng/µl)	–	6 µl	6 µl
Total	**120 µl**	**120 µl**	**120 µl**

4) Add 6 µl of **IDO Assay Buffer** to the wells designated "Blank."

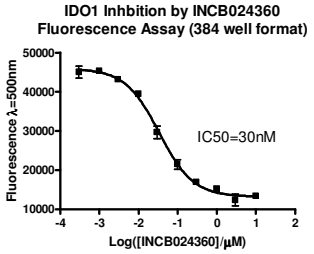
5) Initiate reaction by adding 6 µl of diluted **IDO1** prepared as described above to the wells labeled "Positive Control," and "Test Inhibitor." Incubate at room temperature for 1 hour.

6) Add 16 µl **Fluorescence Solution** to each well. Seal the plate and incubate at 37ºC for four hours. Allow plate to cool for at least 10 minutes.

7) Unseal the plate. For best results, centrifuge plate to reduce condensation on the film. Inspect plate for wells with large changes in volume. Wells with significant changes

should be excluded from data analysis. Measure fluorescence in a fluorimeter capable of excitation at 400 nm and emission at 510 nm. Subtract "Blank" value from all other values.

EXAMPLE OF ASSAY RESULTS:



Inhibition of IDO1 activity by INCB024360 (Cat. #27338), measured using the *IDO1 Fluorogenic Inhibitor Screening Assay Kit (384 well format)*, Catalog #72047. *Data shown is lot-specific.*

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
IDO1 Cellular Activity QuickDetect™ Supplements	62000-1	100 rxns
N-formylkynurenine	73000	2 mg
NLG919	27337-1	10 mg
INCB024360	27338-1	10 mg
IDO1 Reaction Solution	73001	10 ml
IDO1 Assay Buffer	73002	1 ml
IDO2 Reaction Solution	73003	10 ml
IDO2 Assay Buffer	73004	1 ml

AMSBIO| www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85