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ab273304 Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitor Assay Kit

View Kit datasheet: https://www.abcam.com/ab273304
(use https://www.abcam.cn/ab273304 for china, or
https://www.abcam.co.jp/ab273304 for Japan)

For the determination of IDO1 inhibition by drugs or novel chemical entities.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitor Assay Kit (ab273304) enables rapid screening of test compounds for modulation of IDO1 activity.

Unlike conventional colorimetric IDO1 assays based upon UV absorbance, the assay uses a fluorogenic developer that selectively reacts with NFK to produce a highly fluorescent product detectable in the visible range (Ex/Em = 402/488 nm).

This ensures a high signal-to-background ratio and little interference due to short wavelength absorbance by test compounds. The assay is highly sensitive, has a simple no-wash protocol and is high-throughput adaptable. The kit contains a complete set of reagents sufficient for performing 100 reactions in a 96-well plate format.

2. Protocol Summary

Prepare Test Compounds as directed.

Prepare all reagents as directed.

Prepare Assay reaction wells; No Inhibitor Control, Test Compound, Background Contol and Positve Inhibition Control.

Incubate plate for 10 mins at RT.

Prepare Substrate Solution during this incubation.

Add Substrate Solution (10 µL) Incubate for 45 mins at 37°C in the dark with gentle shaking.

Add 50 µL Fluorogenic developer solution to each well and seal plate tightly. Incubate in the dark for 3 hours at 50°C.

Allow the plate to cool to RT for at least 1 hour. Briefly centrifuge plate. Measure fluorescence (Ex/Em = 402/488 nm) in end-point mode.

Calculate % relative inhibition using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
IDO1 Assay Buffer	50 mL	-20°C	-20°C
100X Antioxidant Mix	1 vial	-20°C	-20°C
IDO1 Substrate	1 vial	-20°C	-20°C
IDO1 Inhibitor	1 vial	-20°C	-20°C
Fluorogenic Developer Solution	5 mL	-20°C	-20°C
Human IDO1	1 vial	-20°C	-20°C
Microplate sealing film	1 unit	-20°C	-20°C or RT

PLEASE NOTE: IDO1 Inhibitor was previously labelled as IDO1 Inhibitor (IDO5L) (Lyophilized), and IDO1 Substrate as IDO1 Substrate (L-Tryptophan) (Lyophilized), and Human IDO1 as Recombinant Human IDO1 (Lyophilized). The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well fluorescence microplate reader
- Precision multi-channel pipette and reagent reservoir
- Anhydrous (reagent grade) DMSO
- Black 96-well plate with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **IDO1 Assay Buffer:**

Allow to thaw to room temperature before use. Store at -20°C, protected from light.

9.2 **100X Antioxidant Mix:**

Reconstitute with 110 µl IDO1 Assay Buffer and thoroughly pipette up and down to obtain a 100X stock solution. Aliquot as desired and store aliquots at -20°C, protected from light. Avoid repeated freeze/thaw cycles

9.3 **IDO1 Substrate:**

Reconstitute with 110 µl IDO1 Assay Buffer and vortex to obtain a 10 mM stock solution. Aliquot as desired and store aliquots at -20°C, protected from light. Avoid repeated freeze/thaw cycles.

9.4 **IDO1 Inhibitor:**

Reconstitute with 55 µl anhydrous DMSO and vortex to obtain a 1 mM stock solution (1000X final concentration). Aliquot and store at -20°C, protected from light. Stable for at least three freeze/thaw cycles

9.5 **Fluorogenic Developer solution:**

Allow to warm to room temperature before use. Promptly close and retighten cap after use to prevent evaporation or adsorption of airborne moisture. Store at -20°C, protected from light.

9.6 **Human IDO1:**

Do not open or reconstitute until ready to use. Reconstitute with 1.1 ml IDO1 Assay Buffer and aliquot as desired. Store aliquots at -20°C and use within two months. Avoid repeated freeze/thaw cycles and keep thawed aliquots on ice while in use (once thawed, aliquots should be used within 2 hours).

10. Test Sample Preparation

- For each test compound (TC), dissolve in proper solvent to produce a stock solution and prepare a 10X working solution by diluting the stock solution in IDO1 Assay Buffer.

- To determine IC_{50} values for TCs, 10X solutions should be prepared in a range of concentrations in order to generate a multi-point dose-response curve (the amount of organic solvent should be the same for all test concentrations).

- Organic solvent concentration should be minimized to avoid impacting enzyme activity (DMSO has little effect on IDO1 activity at a final concentration of ≤0.5%).

- For higher concentrations or solvents other than DMSO, we recommend preparing a solvent control (SC) well with the same final concentration of solvent used to solubilize TCs and using this well to define 100% activity if different from no inhibitor control well(s).

11. Assay Procedure

Thaw all reagents thoroughly and mix gently.

Reaction Premix:

11.1 Prepare a 2X Reaction Premix by diluting the 100X Antioxidant Mix with IDO1 Assay Buffer to a 1:50 ratio.

11.2 Prepare enough 2X Reaction Premix to add 50 µL to each well. E.g. for 10 wells:

Component	Volume
IDO1 Assay Buffer	490 µL
100X Antioxidant Mix	10 µL

Δ Note: Remember to account for any control reactions (such as background control, no inhibitor/solvent control and positive inhibition control wells) when calculating the amount of 2X Reaction Premix to prepare.

Assay Layout:

11.3 Set up the assay reactions as in the table below.

11.4 Prepare reaction wells containing Test Compounds, as well as corresponding No Inhibitor Control (which may also serve as a Solvent Control (SC), if desired) and Background Control wells.

11.5 A Positive Inhibition Control well may also be prepared using the IDO1 Inhibitor. Dilute the stock at a 1:100 ratio by adding 10 µl of the reconstituted 1 mM solution to 990 µl IDO1 Assay Buffer, yielding a 10 µM working solution (10X final concentration) and add 10 µl of the 10X solution to each Positive Inhibition Control well.

11.6 Adjust the volume of all TC and control wells to 90 µl with IDO1 Assay Buffer.

	No Inhibitor Control	Test Compound	Background Control	Positive Inhibition Control
Reaction Premix (2X)	50 µL	50 µL	50 µL	50 µL
Test compound (10X)	---	10 µL	---	---
Human IDO1	10 µL	10 µL	---	10 µL
IDO1 inhibitor	---	---	---	10 µL
IDO1 Assay Buffer	30 µL	20 µL	40 µL	20 µL

Δ Note: For solvent control (SC), use 30 µl IDO1 Assay Buffer containing the appropriate solvent at 3.33X final concentration.

11.7 Adjust the volume of all Test Compound and Control wells to 90 µL with IDO1 Assay Buffer.

11.8 Pre-incubate the plate for 10 minutes at room temperature. Prepare Substrate Solution during this incubation.

Substrate Solution:

11.9 Prepare IDO1 Substrate Solution by adding 100 µL of the reconstituted 10 mM L-tryptophan solution to 900 µL IDO1 Assay Buffer, generating a 1 mM solution (10X final concentration).

11.10 Add 10 µL of Substrate Solution to each well for a final reaction volume of 100 µl/well.

11.11 Incubate the plate at 37°C in the dark for 45 minutes with gentle shaking.

Measurement:

11.12 Add 50 µL of the Fluorogenic Developer solution to each well and tightly seal the plate using the plate seal.
11.13 Incubate the plate at 50°C in the dark for 3 hours, then allow the plate to cool to room temperature for at least 1 hour.

Δ Note: The fluorescent signal generated by the Fluorogenic Developer Solution is stable for 8-12 hours after the incubation at 50°C, as long as the plate remains sealed and protected from light.

11.14 Briefly centrifuge the plate before unsealing and measure the fluorescence at (Ex/Em = 402/488 nm) in end-point mode.

12. Calculations

12.1 For each reaction well, including no inhibitor/solvent control and positive inhibition controls, subtract the fluorescence intensity of the background control well to determine background-corrected fluorescence (denoted by F).
12.2 Calculate percent inhibition versus no inhibitor/solvent control (SC) due to the test compound (TC) or IDO5L positive inhibition control using the following equation:

$$Relative\ inhibiton\ \% = \frac{F_{SC} - F_{TC}}{F_{SC}} X\ 100\%$$

F = Background corrected fluorescence
SC = No inhibitor control
TC = Test compound

13. Typical Data

Data provided **for demonstration purposes only.**

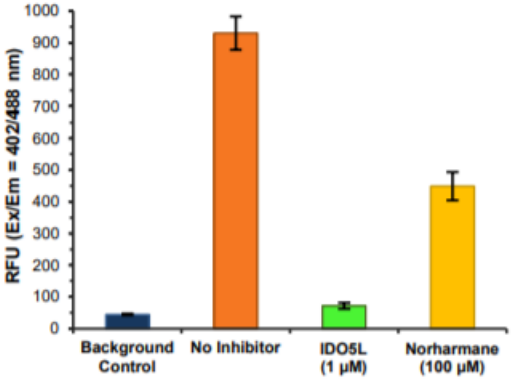

Figure 1. Measurement of IDO1 inhibition in presence and absence of 1 µM of IDO5L (a potent, highly selective competitive IDO1 inhibitor) and 100 µM of norharmane (a natural product that acts as a weak IDO1 inhibitor). The no inhibitor reaction contained assay buffer with 0.1% DMSO (v/v) as a solvent control.

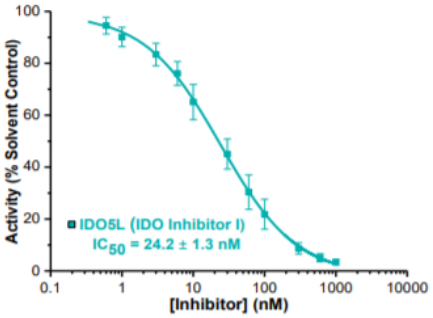

Figure 2. Dose-response curve for IDO1 inhibition by the included selective IDO1 Inhibitor. The IC50 value (24.2 ± 1.3 nM) was derived by 4-parameter logistic curve fitting with each point representing the mean ± EM of at least 3 replicates. All assays were performed according to the kit protocol.

14. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)