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ab235936 Indoleamine 2,3-Dioxygenase 1 (IDO1) Activity Assay Kit

For the measurement of IDO1 activity in mammalian tissues and cell lines.

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

Table of Contents

1. Overview

Indoleamine 2,3-Dioxygenase 1 (IDO1) Activity Assay Kit (ab235936) enables IDO1 activity to be easily determined in mammalian tissues and cell lines. The assay uses a fluorogenic developer that selectively reacts with *N*-formylkynurenine (NFK) to produce a highly fluorescent product (Ex/Em = 402/88 nm), ensuring a high signal-to-background ratio. The kit also includes a highly selective IDO1 inhibitor for verification of enzyme activity in biological matrices. The assay has a simple no-wash protocol, is high-throughput adaptable and can detect down to 0.2 mU of IDO1 activity or 200 pmole NFK.

Prepare samples

⇩

Prepare Standard

⇩

Prepare Reaction Premix and add to sample, positive inhibition control, background control and IDO1 positive control wells.

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Prepare IDO1 Substrate solution and add to sample, positive inhibition control, background control and IDO1 positive control wells. Incubate in the dark for 45 minutes at 37 °C

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Incubate plate with Fluorogenic Developer Solution at 50°C in the dark for 3 hours with gentle shaking.

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Allow to cool and measure fluorescence (Ex/Em = 402/488 nm)

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
IDO1 Assay Buffer	50 mL	-20°C/4°C	-20°C/4°C
100X Antioxidant Mix	1 vial	-20°C	-80°C
N-formylkynurenine Standard	1 vial	-20°C	-80°C
IDO1 Substrate	1 vial	-20°C	-80°C
IDO1 Inhibitor	1 vial	-20°C	-20°C
Fluorogenic Developer Solution	5 mL	-20°C	+4°C
Recombinant Human IDO1	1 vial	-20°C	-80°C
Microplate Sealing Film	1 Unit	-20°C	-20°C

3. Materials Required, Not Supplied

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

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

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 IDO1 Assay Buffer

1. Ready to use as supplied.
2. Allow to thaw to room temperature before use.

5.2 100X Antioxidant Mix

1. Reconstitute with 110 µL IDO1 Assay Buffer and thoroughly pipette up and down to obtain a 100X stock solution.

5.3 *N*-formylkynurenine Standard

1. Reconstitute with 55 µL anhydrous DMSO and vortex until fully dissolved to obtain a 1 mM stock solution.

5.4 IDO1 Substrate

1. Reconstitute with 110 µL IDO1 Assay Buffer and vortex to obtain a 10 mM stock solution.

5.5 IDO1 Inhibitor

1. Reconstitute with 55 µL anhydrous DMSO and vortex to obtain a 1 mM stock solution (100X final concentration).

5.6 Fluorogenic Developer Solution

1. Ready to use as supplied.
2. Allow to warm to room temperature before use.
3. Promptly close and retighten cap after use to prevent evaporation or adsorption of airborne moisture.

5.7 Recombinant Human IDO1

1. Do not open or reconstitute until ready to use.
2. Reconstitute with 110 µL IDO1 Assay Buffer and aliquot as desired.
3. Keep thawed aliquots on ice while in use.

6. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

6.1 Mammalian tissue or cells:

1. Homogenize mammalian tissue (~50 mg) or pelleted, pre-washed cells (~5 x 10^6) in 500 µL ice-cold IDO1 Assay Buffer with a Dounce homogenizer.
2. Vortex the homogenate for 30 seconds, incubate on ice for 5 minutes and centrifuge (10,000 x *g*, 15 minutes, 4°C).
3. Collect the supernatant.
4. Keep on ice until use.

Δ Note: Lysates can also be stored at -80°C for future experiments.

Δ Note: We recommend measuring protein concentration using the Bradford reagent or a comparable protein assay.

Δ Note: We recommend using a protease inhibitor cocktail PMSF to prevent IDO1 degradation.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Reaction Preparation

1. Prepare a 2X Reaction Premix by diluting the 100X Antioxidant Mix in IDO1 Assay Buffer at a 1:50 ratio. Make a sufficient amount of 2X Reaction Premix to add 50 µL to each reaction well.
2. Set up the assay reaction wells, positive inhibition control, background control and IDO1 positive control according to the table below, using a black 96-well microplate.
3. For the positive inhibition control: dilute the IDO5L 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).
4. For other test ligands: dissolve ligands in proper solvent to produce a stock solution and prepare a 10X working solution in IDO1 Assay Buffer. The final concentration of organic solvent should be minimized to avoid impacting IDO1 activity (DMSO has little effect on activity at a final concentration of ≤1%).
5. Adjust the volume of all sample and control wells to 90 µL with IDO1 Assay Buffer.

Component	Test Sample (µL)	Positive Inhibition control /Test Ligand (µL)	Background Contol (µL)	Positive Control (µL)
Reaction Premix (2X)	50	50	50	50
Test Sample	1-30	1-30	-	-
Recombinant Human IDO1	-	-	-	10
IDO5L (10X) or Test Ligand (10X)	-	10	-	-
IDO1 Assay Buffer	To 90	To 90	40	30

6. 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).
7. Add 10 µL of the 1 mM IDO1 Substrate solution to each assay well, for a final reaction volume of 100 µL/well.
8. Incubate the plate at 37°C in a dark environment for 45 minutes (we recommend incubating with gentle shaking to ensure adequate mixing of well contents).

7.2 Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute the *N*-formylkynurenine Standard at a 1:10 ratio (i.e. add 50 µL of 1 mM solution to 450 µL IDO1 Assay Buffer).
2. Using *N*-formylkynurenine standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard#	NFK Standard (µL)	IDO1 Assay Buffer (µL)	Final volume standard in well (µL)	End amount of NFK in well (pmole/well)
1	0	100	100	0
2	2	98	100	200
3	4	96	100	400
4	6	94	100	600
5	8	92	100	800
6	12	88	100	1200
7	16	84	100	1600
8	20	80	100	2000

Each dilution has enough standard to set up duplicate readings (2 x 100 µL).

7.3 Measurement

1. Add 50 µL of the Fluorogenic Developer Solution to each well (including standard curve wells) and tightly seal the plate with the sealing film.
2. Incubate the plate at 55°C in the dark for 3 hours with gentle shaking, then allow to cool to room temperature for 1 hour.
3. Briefly centrifuge the plate. Carefully remove the plate sealing film and measure the fluorescence (Ex/Em = 402/488 nm) in end-point mode.

   Δ Note: The fluorescent signal is stable for 8-12 hours after the incubation at 55°C, as long as the plate remains sealed and protected from light.

8. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of NFK.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. For all sample wells, quantify the specific fluorescence (C_S) by subtracting the fluorescence intensity of the background control (F_{BC}) from the fluorescence intensity of the sample (F_S): $C_S = F_S - F_{BC}$.
7. IDO1 metabolic activity is obtained by applying the C_S values to the NFK standard curve to get *B* pmole of L-tryptophan metabolized by IDO1 during the reaction time.

$$IDO1\ Specific\ Activity = \frac{B}{\Delta T x P} = pmole/min/mg = \mu U/mg$$

Where:

B = amount of *N*-formylkynurenine produced in the sample well calculated from standard curve in pmole.

ΔT = reaction time (45 minutes).

P = amount of protein in the well in mg.

9. FAQs / Troubleshooting

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

10. Typical Data

Data provided **for demonstration purposes only**.

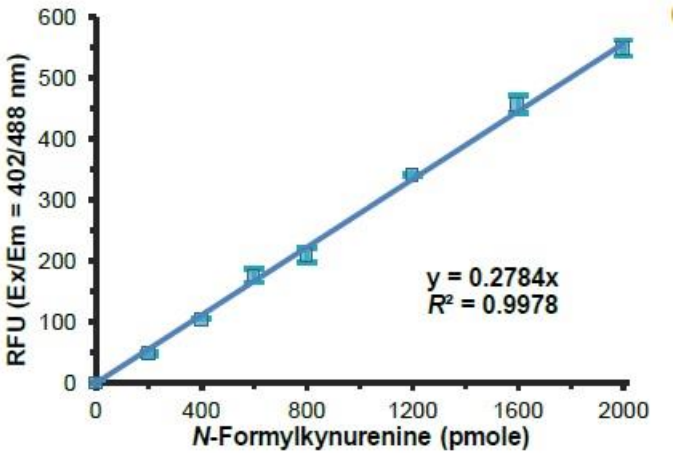

Figure 1. *N*-formylkynurenine (NFK) standard curve: the reaction of 1 mole of NFK with Fluorogenic Developer corresponds to the metabolism of one mole of L-tryptophan by IDO1.

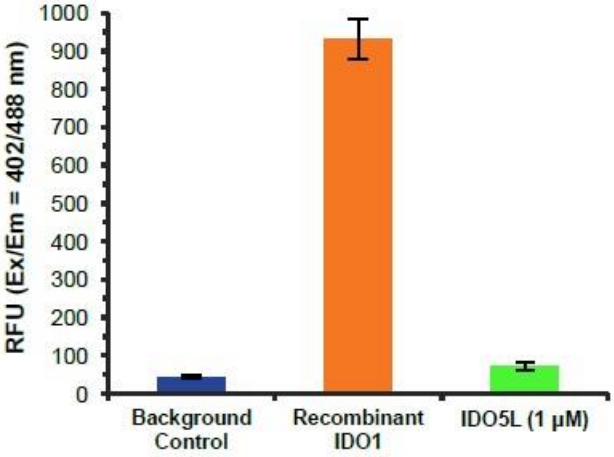

Figure 2. Measurement of IDO1 Positive Control in presence and absence of 1 µM of the included selective inhibitor IDO5L.

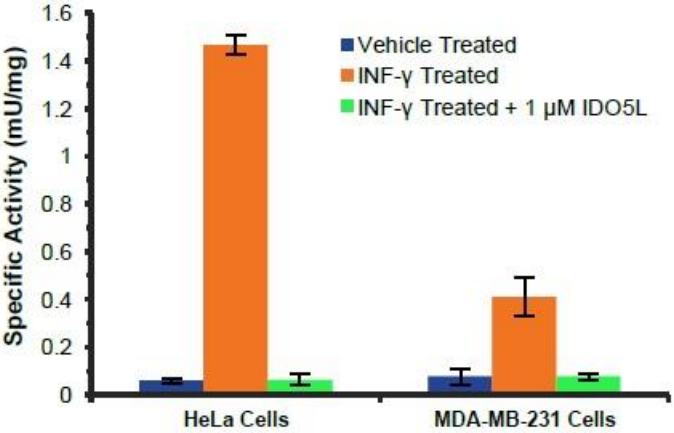

Figure 3. IDO1 activity in lysates (30 µL) of human cancer cell lines stimulated with vehicle (dH_2O) or 100 ng/mL human interferon-γ for 24 hours prior to assay. All assays were performed according to the kit protocol.

11. 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)