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Product Information

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

Catalog Number **MAK356**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Indoleamine 2,3-Dioxygenase 1 (IDO1) is a cytoplasmic hemoprotein that oxidizes tryptophan yielding *N*-formyl-kynurenine (NFK). In mammals, this reaction is the first and rate limiting step in the kynurenine catabolic pathway. IDO1 activity is low under normal physiological conditions, but is dramatically upregulated by proinflammatory cytokines such as interferon-γ. This short-term activation of IDO1 occurs as part of the innate immune response and helps to inhibit the growth of pathogens and parasites. IDO1 activation can also promote host immune tolerance by exerting an immunosuppressive effect. IDO1 expression by tumor cells plays a substantial role in tumor immune tolerance, aiding tumors in evading detection and destruction. Chronic induction of IDO1 expression has been found in cancer patients and is correlated with negative prognosis. IDO1 has thus become an attractive pharmacological target for development of novel antineoplastics and adjuvants to increase the efficacy of conventional chemotherapy.

The Indoleamine 2,3-Dioxygenase 1 Activity Assay Kit enables IDO1 activity to be easily determined in mammalian tissues and cell lines. The assay uses a fluorogenic developer that selectively reacts with NFK to produce a highly fluorescent product (λ_{ex} = 402 nm/ λ_{em} = 488 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.

The kit is suitable for the assessment of native or recombinant IDO1 activity and the screening of drugs and novel ligands for induction or inhibition of cellular IDO1 activity in mammalian tissues expressing IDO1, cultured cells treated with IDO1-inducing cytokines (e.g. interferon-γ), recombinant IDO1 enzyme preparations, and IDO1-expressing heterologous cells.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

IDO1 Assay Buffer Catalog Number MAK356A	50 mL
Antioxidant Mix (100×) Catalog Number MAK356B	1 vial
N-Formylkynurenine Standard Catalog Number MAK356C	1 vial
IDO1 Substrate (L-tryptophan) Catalog Number MAK356D	1 vial
IDO1 Inhibitor (IDO5L) Catalog Number MAK356E	1 vial
Fluorogenic Developer Solution Catalog Number MAK356F	5 mL
Recombinant Human IDO1 Catalog Number MAK356G	1 vial

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flatbottom 96 well plates
- Fluorescence multiwell plate reader
- Dimethyl Sulfoxide (DMSO), anhydrous (Catalog Number 276855)
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Bradford Reagent, (Catalog Number B6916)
- Protease Inhibitor Cocktail (Catalog Number P8340)
- 0.1 M PMSF, (Catalog Number 93482)

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions.
IDO1 Assay Buffer: Allow to thaw to room temperature, protected from light, prior to use.

Antioxidant Mix (100×): Reconstitute with 110 µL of IDO1 Assay Buffer and thoroughly pipette up and down to obtain a 100× stock solution. Aliquot as desired and store aliquots at –80 °C, protected from light. Avoid repeated freeze/thaw cycles.

N-Formylkynurenine Standard: Reconstitute with 55 µL of anhydrous DMSO and vortex until fully dissolved to obtain a 1 mM stock solution. Aliquot as desired and store aliquots at –80 °C, protected from light. Avoid repeated freeze/thaw cycles.

IDO1 Substrate (L-tryptophan): Reconstitute with 110 µL of IDO1 Assay Buffer and vortex to obtain a 10 mM stock solution. Aliquot as desired and store aliquots at –80 °C, protected from light. Avoid repeated freeze/thaw cycles.

IDO1 Inhibitor (IDO5L): Reconstitute with 55 µL of anhydrous DMSO and vortex to obtain a 1 mM stock solution (1,000× final concentration). Aliquot and store at –20 °C, protected from light. Stable for at least 3 freeze/thaw cycles.

Fluorogenic Developer Solution: Allow to warm to room temperature prior to use. Promptly close and retighten cap after use to prevent evaporation or adsorption of airborne moisture. Store at 4 °C, protected from light.

Recombinant Human IDO1: Do not open or reconstitute until ready to use. Reconstitute with 110 µL of IDO1 Assay Buffer and aliquot as desired. Store aliquots at –80 °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).

Procedure
Sample Preparation
1. Use of a protease inhibitor cocktail containing PMSF to prevent IDO1 degradation is suggested.
2. Homogenize mammalian tissue (~50 mg) or pelleted, pre-washed cells (~5×10^6) in 500 µL of ice-cold IDO1 Assay Buffer with a Dounce homogenizer.
3. Vortex the homogenate for 30 seconds.
4. Incubate on ice for 5 minutes and centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Collect the supernatant.
6. Keep on ice until use. Lysates can also be stored at –80 °C for future experiments.
7. It is suggested to measure the protein concentration using Bradford Reagent or a comparable protein assay.

Standard Curve Preparation
Prepare a 0.1 mM solution by diluting the *N*-Formyl-kynurenine Standard 10-fold with IDO1 Assay Buffer (i.e., add 50 µL of 1 mM stock solution to 450 µL of IDO1 Assay Buffer). Prepare *N*-Formylkynurenine (NFK) Standards in desired wells of a black 96 well plate according to Table 1. Mix well.

Table 1.
Preparation of *N*-Formylkynurenine (NFK) Standards

Well	0.1 mM Premix	IDO1 Assay Buffer	NFK (pmole/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	200
3	4 µL	96 µL	400
4	6 µL	94 µL	600
5	8 µL	92 µL	800
6	12 µL	88 µL	1,200
7	16 µL	84 µL	1,600
8	20 µL	80 µL	2,000

Assay Reaction
1. Prepare a 2× Reaction Premix by diluting the 100× Antioxidant Mix 50-fold in IDO1 Assay Buffer. Make a sufficient amount of 2× Reaction Premix to add 50 µL to each reaction well. Remember to account for any control reactions (such as background control, inhibitor, and positive control wells) when calculating the amount of 2× Reaction Premix to prepare.
2. Set up the assay reaction wells, positive inhibition control, background control (BC), and IDO1 positive control according to Tables 2 and 3, in a black 96 well microplate.

Table 2.
Preparation of Assay Reaction Wells and Positive Inhibition Control/Test Ligand

Reagent	Test Sample	Plus Inhibitor/ Test Ligand
Reaction Premix (2×)	50 µL	50 µL
Test Sample	1–30 µL	1–30 µL
Recombinant Human IDO1	–	–
IDO5L (10×) or Test Ligand (10×)	–	10 µL
IDO1 Assay Buffer	To 90 µL	To 90 µL

Table 3.
Preparation of background control and IDO1 positive control

Reagent	Background Control	Positive Control
Reaction Premix (2×)	50 µL	50 µL
Test Sample	–	–
Recombinant Human IDO1	–	10 µL
IDO5L (10×) or Test Ligand (10×)	–	–
IDO1 Assay Buffer	40 µL	30 µL

3. For the positive inhibition control, dilute the IDO1 Inhibitor (IDO5L) 1 mM stock solution 100-fold by adding 10 µL of the reconstituted 1 mM solution to 990 µL of IDO1 Assay Buffer, yielding a 10 µM working solution (10× final concentration). For other test ligands: dissolve ligands in proper solvent to produce a stock solution and prepare a 10× 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%). Adjust the volume of all sample and control wells to 90 µL with IDO1 Assay Buffer.
4. Prepare IDO1 Substrate solution by adding 100 µL of the reconstituted 10 mM L-tryptophan solution to 900 µL of IDO1 Assay Buffer, generating a 1 mM solution (10× final concentration). Add 10 µL of the 1 mM solution to each assay well, for a final reaction volume of 100 µL/well. Incubate the plate at 37 °C in a dark environment for 45 minutes (gentle shaking of the plate during incubation to ensure adequate mixing of well contents is suggested).

Measurement

Add 50 µL of the Fluorogenic Developer Solution to each well (including standard curve wells) and tightly seal the plate with the sealing film. Incubate the plate at 45 °C in the dark for 3 hours with gentle shaking, then allow plate to cool to room temperature for 1 hour. Briefly centrifuge the plate. Carefully remove the plate sealing film and measure the fluorescence (λ_{ex} = 402 nm/λ_{em} = 488 nm) in end-point mode. The fluorescent signal is stable for 8–12 hours after the incubation at 45 °C, as long as the plate remains sealed and protected from light.

Results

1. For the NFK standard curve, subtract the fluorescence intensity of the 0 pmole/well blank from the other NFK standard wells and plot the standard curve.
2. 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):

$$C_S = F_S - F_{BC}.$$

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.

Indoleamine 2,3-Dioxygenase 1 (IDO1) Specific Activity (pmole/min/mg or μU/mg) =

$$B/(\Delta T \times P)$$

where:
B= the amount of *N*-formylkynurenine produced, calculated from the standard curve (in pmole)
ΔT= the reaction time (in minutes)
P= the amount of protein in the well (in mg)

Unit Definition

One unit of IDO1 activity is the amount of enzyme that generates 1 μmole of detected *N*-formylkynurenine per minute by oxidative metabolism of 1 μmole L-tryptophan at 37 °C.

Figure 1.
Typical *N*-Formylkynurenine (NFK) Standard Curve

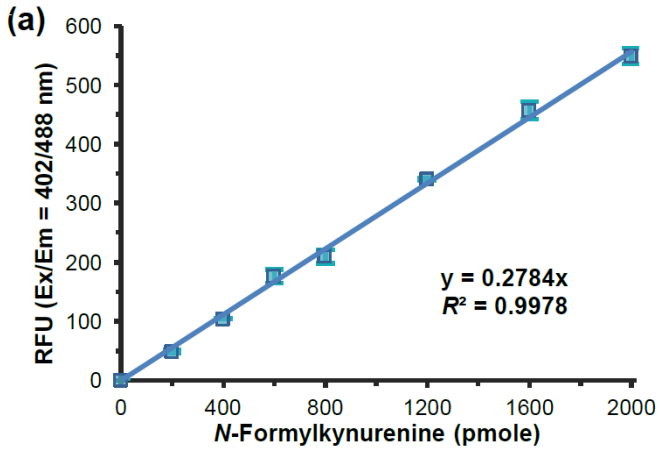


Figure 2.
Measurement of IDO1 Positive Control in presence and absence of 1 μM of the included selective inhibitor IDO5L

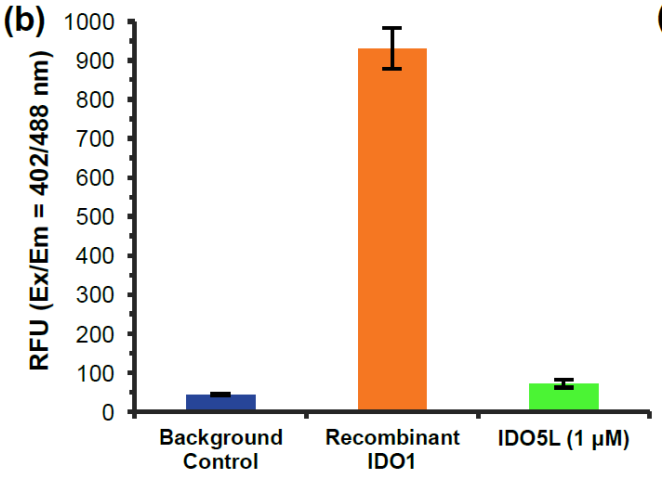
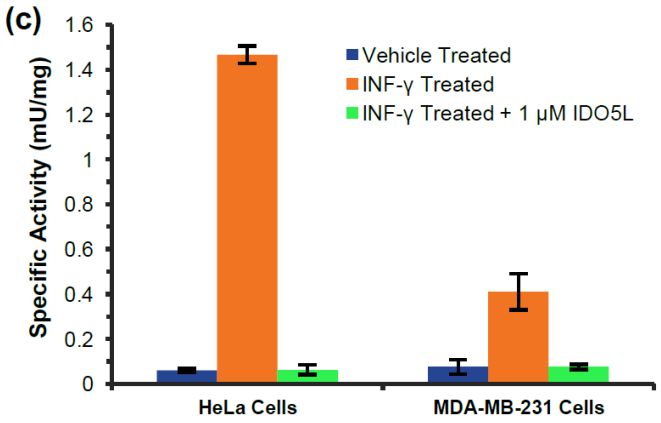


Figure 3.
IDO1 Activity



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

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