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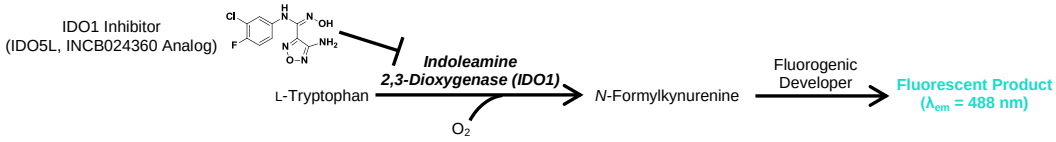
Indoleamine 2,3-Dioxygenase 1 (IDO1) Activity Assay Kit

rev 06/20

(Catalog # K972-100; 100 Reactions; Store at -20°C)

I. Introduction:

Indoleamine 2,3-Dioxygenase 1 (IDO1, EC 1.13.11.52) is a cytoplasmic hemoprotein that oxidizes tryptophan yielding *N*-formylkynurenine (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 increased IDO1 activity 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. **BioVision's IDO1 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 (Ex/Em = 402/488 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.



II. Applications:

- Assessment of native or recombinant IDO1 activity in lysates prepared from tissues and cells.
- Screening of drugs and novel ligands for induction or inhibition of cellular IDO1 activity.

III. Sample Types:

- Mammalian tissues expressing IDO1
- Cultured cells treated with IDO1-inducing cytokines (*e.g.* interferon-γ)
- Recombinant IDO1 enzyme preparations and IDO1-expressing heterologous cells

IV. Kit Contents:

Components	K972-100	Cap Code	Part Number
IDO1 Assay Buffer	50 ml	WM	K972-100-1
Antioxidant Mix (100X)	1 vial	Green	K972-100-2
N-formylkynurenine Standard	1 vial	Yellow	K972-100-3
IDO1 Substrate (L-tryptophan)	1 vial	Red	K972-100-4
IDO1 Inhibitor (IDO5L)	1 vial	Amber	K972-100-5
Fluorogenic Developer Solution	5 ml	NM	K972-100-6
Recombinant Human IDO1	1 vial	Violet	K972-100-7
Microplate Sealing Film	1 film	—	K972-100-8

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Prior to use, store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay procedure.

a. IDO1 Assay Buffer: Allow to thaw to room temperature before use. Store at 4°C or -20°C, protected from light.

b. Antioxidant Mix (100X): 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 -80°C, protected from light. Avoid repeated freeze/thaw cycles.

c. *N*-formylkynurenine Standard: Reconstitute with 55 µl 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.

d. IDO1 Substrate (L-tryptophan): Reconstitute with 110 µl 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.

e. IDO1 Inhibitor (IDO5L): 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 3 freeze/thaw cycles.

f. 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 4°C, protected from light.

g. Recombinant Human IDO1: Do not open or reconstitute until ready to use. Reconstitute with 110 µl 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*).

VII. Indoleamine 2,3-Dioxygenase 1 (IDO1) Activity Assay Protocol:

1. **Sample Preparation:** 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 (Cat. #1998 or equivalent). Vortex the homogenate for 30 sec, incubate on ice for 5 min and centrifuge (10,000 x *g*, 15 min, 4°C). Collect the supernatant. Keep on ice until use (*lysates can also be stored at -80°C for future experiments*). We recommend measuring protein concentration using the Bradford reagent (Cat. #K810) or a comparable protein assay.

   Note: We recommend using a protease inhibitor cocktail containing PMSF (*e.g.* Cat. #K271) to prevent IDO1 degradation.

2. **Assay Reaction Preparation:**

   a. 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. *Remember to account for any control reactions (such as background control, inhibitor and positive control wells) when calculating the amount of 2X Reaction Premix to prepare*.

   b. 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. 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). 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%). Adjust the volume of all sample and control wells to 90 µl with IDO1 Assay Buffer.

	Test Sample	+ Inhibitor/Test Ligand	Background Control	Positive Control
Reaction Premix (2X)	50 µl	50 µl	50 µl	50 µl
Test Sample	1–30 µl	1–30 µl	—	—
Recombinant Human IDO1	—	—	—	10 µl
IDO5L (10X) or Test Ligand (10X)	—	10 µl	—	—
IDO1 Assay Buffer	to 90 µl	to 90 µl	40 µl	30 µl

   c. 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). 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 min (we recommend incubating with gentle shaking to ensure adequate mixing of well contents).

3. **Standard Curve Preparation:** 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). Add 0, 2, 4, 6, 8, 12, 16 and 20 µl of the diluted Standard into a series of wells in the assay plate, yielding 0, 200, 400, 600, 800, 1200, 1600 and 2000 pmole/well *N*-formylkynurenine (NFK). Adjust the volume of each well to 100 µl with IDO1 Assay Buffer.

4. **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 hrs with gentle shaking. Allow cooling to RT for 1 hr and briefly centrifuge the plate. Carefully remove the plate sealing film and measure the fluorescence (Ex/Em = 402/488 nm) in end-point mode. *The fluorescent signal is stable for 8-12 hrs after the incubation at 45°C, as long as the plate remains sealed and protected from light.*

5. **Calculation:** 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. 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.

$$\textbf{Indoleamine 2,3-Dioxygenase 1 (IDO1) Specific Activity} = \frac{B}{\Delta T \times P} = \textbf{pmole/min/mg = µU/mg}$$

Where: B is the amount of *N*-formylkynurenine produced, calculated from the Standard Curve (in pmole)
ΔT is the reaction time (45 minutes)
P is 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 min by oxidative metabolism of 1 µmole L-tryptophan at 37°C.

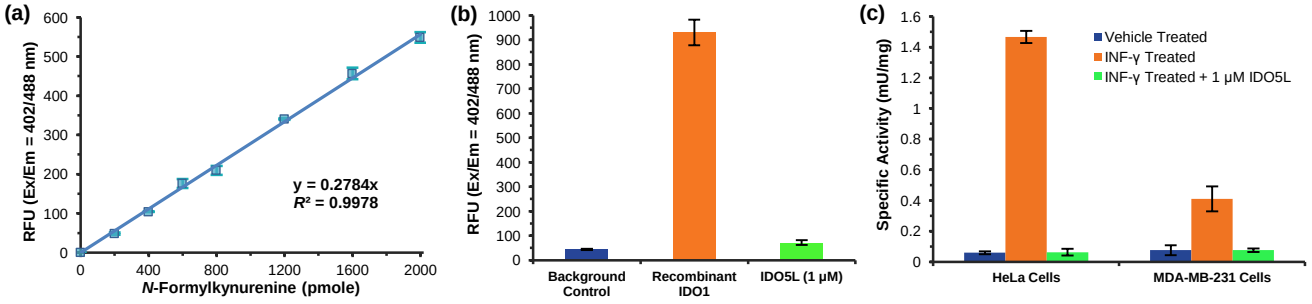


Figure: (a) *N*-formylkynurenine (NFK) standard curve: the reaction of 1 mole of NFK with Fluorogenic Developer corresponds to the metabolism of one mole of L-tryptophan by IDO1. **(b)** Measurement of IDO1 Positive Control in presence and absence of 1 µM of the included selective inhibitor IDO5L. **(c)** IDO1 activity in lysates (30 µl) of human cancer cell lines stimulated with vehicle (dH_2O) or 100 ng/ml human interferon-γ (Cat. #4116) for 24 hours prior to assay. All assays were performed according to the kit protocol.

VIII. RELATED PRODUCTS:

Interferon-γ Human Recombinant (4116) IDO1 Inhibitor Screening Kit (KXXX)

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