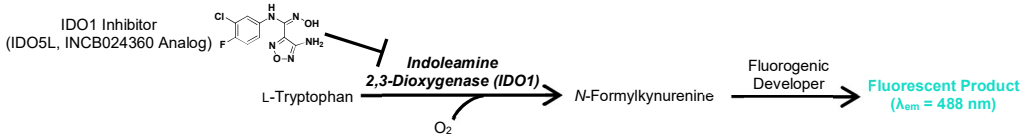
Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitor Screening Kit (#BN01134)

(Catalog # BN01134; 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 patients with many different types of cancer and increased IDO1 activity is correlated with more extensive disease spread and worsening prognosis. IDO1 has thus become an attractive pharmacological target for development of novel antineoplastics and adjuvants to increase the efficacy of conventional chemotherapy. Assay Genie's IDO1 Inhibitor Screening Kit 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.



II. Applications:

- Screening and characterization of drugs and novel chemical entities for inhibition or induction of IDO1 activity.
- Development of structure-activity relationship (SAR) models to predict IDO1 selectivity of compounds.

III. Kit Contents:

Components	BN01134	Cap Code	Part Number
IDO1 Assay Buffer	50 ml	WM	BN01134-1
Antioxidant Mix (100X)	1 vial	Green	BN01134-2
IDO1 Substrate (L-Tryptophan)	1 vial	Red	BN01134-3
IDO1 Inhibitor (IDO5L)	1 vial	Amber	BN01134-4
Fluorogenic Developer Solution	5 ml	NM	BN01134-5
Recombinant Human IDO1	1 vial	Violet	BN01134-6
Microplate Sealing Film	1 film	—	BN01134-7

IV. 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

V. 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 entire protocol before performing the assay procedure.

- **IDO1 Assay Buffer:** Allow to thaw to room temperature before use. Store at 4°C, protected from light.
- **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.
- **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.
- **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.
- **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.
- **Recombinant 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 -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).

VI. Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitor Screening Protocol:

1. **Test Compound 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).

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 (for example, for 10 reactions, mix 10 µl of the 100X Antioxidant Mix with 490 µl IDO1 Assay Buffer).

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.

b. Set up the assay reactions according to the table below. 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. A positive inhibition control well may also be prepared using the IDO1 Inhibitor (IDO5L). 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. Adjust the volume of all TC and control wells to 90 µl with IDO1 Assay Buffer.

	No Inhibitor/SC	+Test Compound	Background Control	Positive Inhibition Control
Reaction Premix (2X)	50 µl	50 µl	50 µl	50 µl
Test Compound (10X)	—	10 µl	—	—
Recombinant Human IDO1	10 µl	10 µl	—	10 µl
IDO1 Inhibitor IDO5L (10X)	—	—	—	10 µl
IDO1 Assay Buffer	30 µl	20 µl	40 µl	20 µl

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

c. Pre-incubate the plate for 10 min at room temperature to allow test compounds to interact with IDO1. The pre-incubation time can be optimized for other test compounds depending on mechanism of action. During the pre-incubation, 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. Measurement: Add 50 µl of the Fluorogenic Developer Solution to each well and tightly seal the plate with the sealing film. Incubate the sealed plate at 45°C in a dark environment for 3 hrs, then allow the plate to cool to room temperature for at least 1 hr. Briefly centrifuge the plate before unsealing. Carefully remove the plate sealing film and measure the fluorescence intensity (Ex/Em = 402/488 nm) of all wells in end-point mode.

Notes:

- The fluorescent signal generated by the Fluorogenic Developer Solution is stable for 8-12 hrs after the incubation at 45°C, as long as the plate remains sealed and protected from light.

4. Calculation: 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***). Calculate percent inhibition versus no inhibitor/solvent control (SC) due to the test compound (TC) or IDO5L positive inhibition control using the following equation:

$$\% \text{ Relative Inhibition} = \frac{F_{SC} - F_{TC}}{F_{SC}} \times 100\%$$

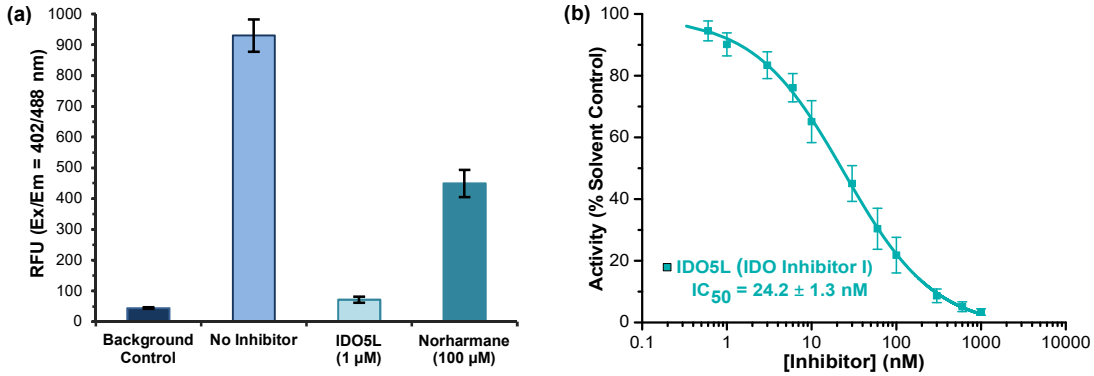


Figure: (a) Measurement of IDO1 inhibition in presence and absence of 1 µM of IDO5L (a potent, highly selective competitive IDO1 inhibitor) and 100 µM of norharmane (a natural product that acts as a weak IDO1 inhibitor). The no inhibitor reaction contained assay buffer with 0.1% DMSO (v/v) as a solvent control. **(b)** Dose-response curve for IDO1 inhibition by the included selective IDO1 Inhibitor IDO5L. The IC_{50} value (24.2 ± 1.3 nM) was derived by 4-parameter logistic curve fitting with each point representing the mean ± SEM of at least 3 replicates. All assays were performed according to the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.