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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

IDO1 Cellular Activity QuickDetect™ Supplements
Catalog: #62000-1

Description: The IDO1 Cellular Activity QuickDetect™ Supplements are designed to complement the hIDO1-HEK293 Recombinant Cell Line (BPS Cat. #60532) or other IDO1-expressing cell lines. This kit contains the cells culture medium supplements necessary for activation of IDO1 and for the analysis and detection of Indoleamine 2,3 dioxygenase 1 (IDO1)-catalyzed conversion of L-tryptophan (L-Trp) to Kynurenine (Kyn). The supplements and the detection reagents, when used as described, allow for indirect measurement of Kyn levels by analyzing absorption at 480 nm.

Background: L-Trp is an essential amino acid necessary for protein synthesis in mammalian cells, and the L-Trp to Kyn pathway is firmly established as a key regulator of innate and adaptive immunity. Catabolism of L-Trp to Kyn maintains an immunosuppressive microenvironment by starving immune cells of L-Trp and releasing degradation products of L-Trp that have immunosuppressive functions. IDO1 is upregulated in many tumors, providing cancer cells with an avenue for immune evasion.

Applications

- Monitor IDO1 pathway activity
- Screen for activators or inhibitors of IDO1 in a cellular context

Format

Component	Amount	Storage
IDO1 Assay Medium Supplement 1	200 µl	4°C
IDO1 Assay Medium Supplement 2	200 µl	-20°C
Detection Reagent	200 mg	Room Temp.

Materials Required but Not Supplied

hIDO1-HEK293 Recombinant Cell Line (BPS Cat. #60532) or other IDO1-expressing cell line and appropriate cell culture medium
INCB024360 (BPS Bioscience #27339) or other IDO1 inhibitor
6.1 N (concentrated) trichloroacetic acid*
17.4 N (concentrated) glacial acetic acid*
**Note: both trichloroacetic acid and acetic acid are strongly corrosive acids; please use gloves and appropriate protective clothing.*

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General Assay Procedure

1) On day 1, seed cells at a density of 30,000 cells in 100 µl of growth medium *without antibiotic* into each well of a tissue culture-treated 96-well plate. Incubate cells at 37°C in a CO_2 incubator overnight.

2) Prepare fresh *Assay Medium* by diluting **IDO1 Assay Medium Supplement 1** 1:100 and **IDO1 Assay Medium Supplement 2** 1:100 into cell culture medium.

3) Dilute the INCB024360 (10 µM) or test compound to the desired concentration in fresh *Assay Medium*. Remove the cell culture medium from transfected cells and replace with 200 µl of *Assay Medium* containing the inhibitor compound. Incubate cells overnight at 37°C in a CO_2 incubator. *Note: The final concentration of DMSO in the cell culture should not exceed 0.3%.*

4) On the next day, remove 140 µl of medium from each well of the cell culture and transfer into a fresh 96-well plate. Add 10 µl of 6.1 N trichloroacetic acid to each well. Incubate the plate at 50°C for 30 min. Centrifuge the plate at 2500 rpm for 10 minutes to remove any sediment. If a plate centrifuge is not available, the liquid can be transferred to a microcentrifuge tube and spun briefly to pellet any solids.

5) Prepare *Detection Reagent Solution* by dissolving **Detection Reagent** at a 50-fold dilution in acetic acid, e.g. 200 mg in 10 ml undiluted acetic acid. Prepare only enough reagent required for the assay.

6) Transfer 100 µl of supernatant to a transparent 96-well plate and mix with 100 µl of fresh *Detection Reagent Solution*. Incubate the plate at room temperature for 10 minutes, then measure absorbance at 480 nm using a microplate reader.

7) Data analysis: The total absorbance (At), without inhibitor treatment, should be set to 100%.The absorbance of cell-free control wells (Ab) in each data set should be defined as 0%. The percent absorbance in the presence of reference inhibitor compound is calculated according to the following equation: % Absorbance = (A-Ab)/(At-Ab), where A= the absorbance in the presence of the compound.

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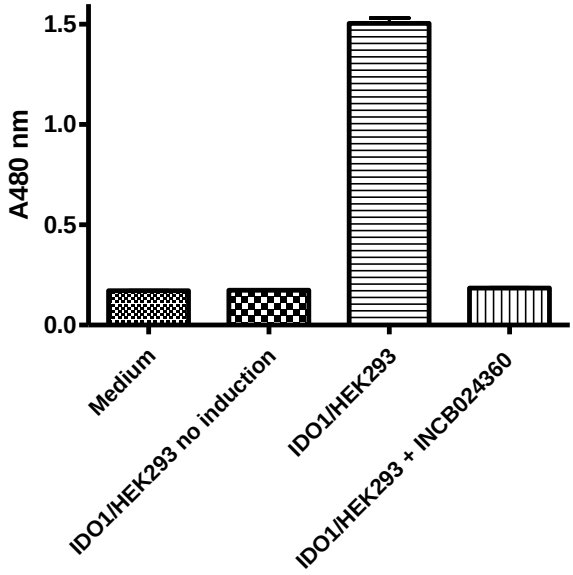
Figure 1. hIDO1-catalyzed Kyn production from L-Trp in expressed in hIDO1-HEK293 Recombinant Cell Line (BPS Cat. #60532).



INCB024360 completely blocks hIDO1 enzyme activity at a concentration of 100 µM. The results are shown as raw absorbance data at 480 nm. Conditions from left to right: medium only (no cells), hIDO1 – HEK293 Cells with no induction plus all assay components, hIDO1 – HEK293 Cells with induction plus all assay components, hIDO1 – HEK293 Cells with induction plus all assay components and INCB024360.

Reference

1. Liu, X., *et al., Blood.* 2010; **115(17):** 3520-3530.

Related Products

Product Name	Catalog #	Size
hIDO1-HEK293 Recombinant Cell Line	60532	2 vials
IDO1 Cell-Based Assay Kit	72031	100 rxns
TDO Cell-Based Assay Kit	72033	100 rxns
IDO1 Inhibitor Screening Assay Kit	72021	96 rxns
IDO2 Inhibitor Screening Assay Kit	72022	96 rxns
TDO Inhibitor Screening Assay Kit	72023	96 rxns
TDO, His-tag	71195	50 µg
IDO1, His-tag	71182	50 µg
IDO2, His-tag	71194	200 µg