Description

The IDO2 Cellular Activity QuickDetect™ Supplements are designed to complement the hIDO2-HEK293 Recombinant Cell Line (BPS Bioscience #60533) or other IDO2-expressing cell lines. This kit contains the cell culture medium supplements necessary for activation of IDO2 and for the analysis and detection of Indoleamine 2,3 dioxygenase 2 (IDO2)-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-tryptophan (L-Trp) is an essential amino acid necessary for protein synthesis in mammalian cells. The L-Trp to kynurenine (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. Additionally, the released degradation products of L-Trp have immunosuppressive functions. Indoleamine 2,3-dioxygenases (IDO1 & IDO2), two of the rate limiting enzymes in this pathway, are upregulated in many tumors and provide cancer cells with an avenue for immune evasion.

Application

- Monitor IDO2 pathway activity
- Screen for compound activity of IDO2 in a cellular context

Materials Provided

Components	Format	Storage
IDO2 Assay Medium Supplement 1	2 x 1 ml	4°C
IDO2 Assay Medium Supplement 2	4 x 500 µl	-20°C
Detection Reagent	2 g	Room Temperature

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Materials Required for Cellular Assay

Name	Ordering Information
hIDO2-HEK293 Recombinant Cell Line or other IDO2-expressing cell line and appropriate cell culture medium	BPS Bioscience #60533
INCB024360 or other IDO2 inhibitor	BPS Bioscience #27339
6.1 N (concentrated) trichloroacetic acid*	
17.4 N (concentrated) acetic acid*	

**Note: both trichloroacetic acid and acetic acid are strongly corrosive acids; please use gloves and appropriate protective clothing.*

Stability

This item will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

A. General Assay Procedure

1. On day 0, seed hIDO2-HEK293 cells at a density of 30,000 cells in 100 µl of Thaw Medium 1 into each well of a tissue culture-treated 96-well plate. Incubate cells at 37°C in a CO_2 incubator overnight. Leave a couple wells empty for use as a background control.
2. Next day (Day 1), prepare **Assay Medium** by diluting Assay Supplement 1 1:50 and Assay Supplement 2 1:100 into Induction Medium.
3. Prepare serial dilutions of INCB024360 in *Assay Medium*. Remove culture medium and treat cells with 200 µl diluted inhibitor. Add 200 µl of **Assay Medium** containing DMSO to cell-free control wells for determining background absorbance. Include wells with cells fed with Thaw Medium 1 as an optional control for determining the basal level of un-induced IDO1 expression. Incubate cells at 37°C in a CO_2 incubator for 72 hours. *Note: The final DMSO concentration should not exceed 0.3%.*
4. On day 4, 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. This is best performed using a 96-well plate or tubes compatible with a thermocycler. Centrifuge the plate at 1300 rcf for 10 minutes to remove any sediment.
5. During the incubation, prepare **Detection Reagent Solution** by dissolving Detection Reagent (Provided in BPS Bioscience #62001) as a 2% solution 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 freshly prepared **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: in the absence of the reference inhibitor the absorbance (At) in each 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.

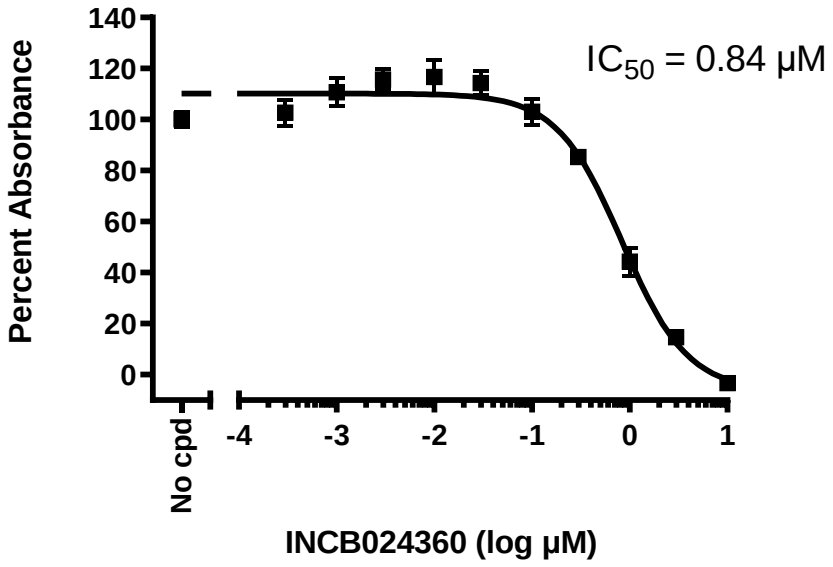

Figure 1. Dose response of hIDO2 activity in hIDO2-HEK293 cells to the reference inhibitor INCB024360. The results are shown as percentage of absorbance. The normalized absorbance for hIDO2 expressed cells without inhibitor treatment was set at 100%. The IC_{50} of INCB024360 is ~ 0.84 µM.

License Disclosure

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
hIDO2-HEK293 Recombinant Cell Line	60533	2 vials
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