Description

The IDO1 Cellular Activity QuickDetect™ Supplements are designed to complement the hIDO1-HEK293 Recombinant Cell Line (BPS Bioscience #60532) or other IDO1-expressing cell lines. This kit contains the cell 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.

Application

- Monitor IDO1 pathway activity
- Screen for compound activity of IDO1 in a cellular context

Materials Provided

Components	Format	Storage
IDO1 Assay Medium Supplement 1	2 x 1 ml	4°C
IDO1 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
hIDO1-HEK293 Recombinant Cell Line or other IDO1-expressing cell line and appropriate cell culture medium	BPS Bioscience #60532
INCB024360 or other IDO1 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 hIDO-HEK293 cells at a density of 30,000 cells in 100 µl of Thaw Medium 1 **(no Geneticin or Blasticidin)** into each well of a tissue culture-treated 96-well plate. Leave a couple wells empty for use as a background control. Incubate cells at 37°C in a CO_2 incubator overnight.
2. The next day (Day 1), remove Thaw Medium 1 and treat the cells with 100 µl of Induction Medium to induce hIDO1 expression.
3. Prepare fresh *Assay Medium* by diluting **IDO1 Assay Medium Supplement 1** 1:100 and **IDO1 Assay Medium Supplement 2** 1:100 into Induction Medium.
4. Prepare serial dilutions of INCB024360 in *Assay Medium*. Remove culture medium and treat with 200 µl of 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 overnight at 37°C in a CO_2 incubator. *Note: The final concentration of DMSO in the cell culture should not exceed 0.3%.*
5. On day 3, 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.
6. Prepare *Detection Reagent Solution* by dissolving **Detection Reagent** 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.
7. 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.
8. 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.

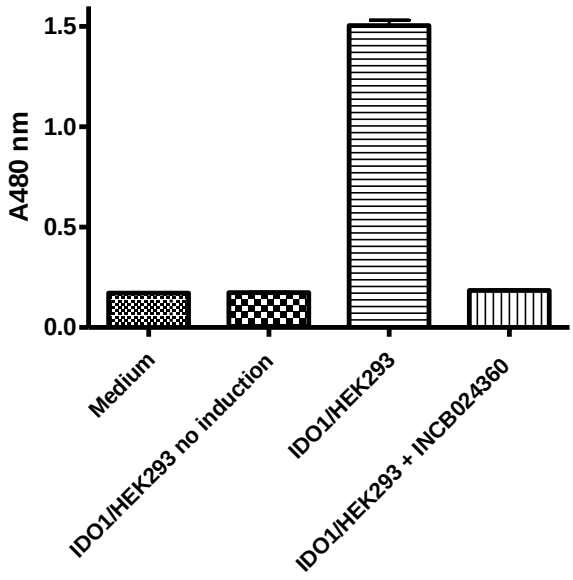

Figure 1. hIDO1-catalyzed Kyn production from L-Trp in hIDO1-HEK293 Recombinant Cell Line (BPS Bioscience #60532). INCB024360 completely blocks hIDO1 enzyme activity at a concentration of 1 µM. The results are shown as raw absorbance data at 480 nm. Conditions from left to right: medium only (no cells), 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.

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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*
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