Description
Recombinant HEK293 cell line expressing tetracycline-inducible human indoleamine 2,3-dioxygenase (IDO2), Genbank accession number NM_194294.

Background
L-tryptophan (L-Trp) is an essential amino acid necessary for protein synthesis in mammalian cells, and 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 and releasing degradation products of L-Trp that have immunosuppressive functions. Indoleamine 2,3-dioxygenases (IDO1 & IDO2), two of the rate limiting enzymes in this pathway, are upregulated in many tumors, providing 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
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Parental Cell Line
HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing
The cell line has been screened to confirm the absence of Mycoplasma species.

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 Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1D	BPS Bioscience #79536

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1D	BPS Bioscience #79536
Doxycycline	MP Biomedicals, #0219504401
INCB024360	BPS Bioscience #27339
6.1 N Trichloroacetic acid	Sigma #T0699
Acetic acid	Sigma #695092
IDO2 Cellular Activity QuickDetect™ Supplements	BPS Bioscience #62001

96-well tissue culture treated assay plate	
96-well plate or tube for thermocycler	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience, #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1D (BPS Bioscience, #79536):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 600 μg/ml of Geneticin, and 5 μg/ml of Blasticidin.

Induction Medium:
Thaw Medium 1 plus 0.2 μg/ml Doxycycline (MP Biomedicals #0219504401).

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin and Blasticidin**).
   Leaving the cells at 37°C for too long will result in rapid loss of viability.
2. Immediately spin down the cells at 300xg for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1 (**no Geneticin and Blasticidin**).
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 (**no Geneticin and Blasticidin**), and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1D (contains **Geneticin and Blasticidin**).

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1D and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1D (**contains Geneticin and Blasticidin**). Seed into new culture vessels at the desired sub-cultivation ratio of 1:2 to 1:6 weekly or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1D and count the cells.
3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

N´-terminal FLAG tagged human IDO2 has been stably integrated into HEK293 cells and its expression can be induced by tetracycline (doxycycline). The tetracycline-inducible expression of hIDO2 was confirmed by Western blotting.

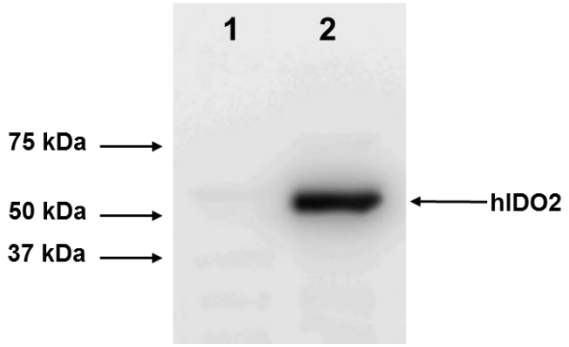

Figure 1. Western Blot of the hIDO2 in hIDO2-HEK293 cells. Western Blot of Tet-Repressor-HEK293 parental cells (lane 1) and hIDO2-HEK293 cells (lane 2). Data shows the tetracycline-inducible expression of hIDO2 in hIDO2-HEK293 cells. Western blot was probed with mouse anti-FLAG antibody. The recombinant human IDO2 comprises 415 amino acids and has a calculated molecular mass of 46.4 KDa. It migrates as an approximately 55 kDa band in SDS-PAGE under reducing conditions.

hIDO2 activity was confirmed by an absorbance-based assay measuring the catalyzed production of kynurenine in cell culture medium. When hIDO2 is expressed in hIDO2-HEK293 cells, it catalyzes L-Trp conversion to Kyn,

which gets released in the assay medium and can be easily detected by a reaction with Ehrlich's reagent, producing a yellow color.

Inhibition of doxycycline-induced hIDO2 activity by INCB024360 in IDO2-HEK293 recombinant cells
Note: We recommend each treatment be set up in at least triplicate.

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.

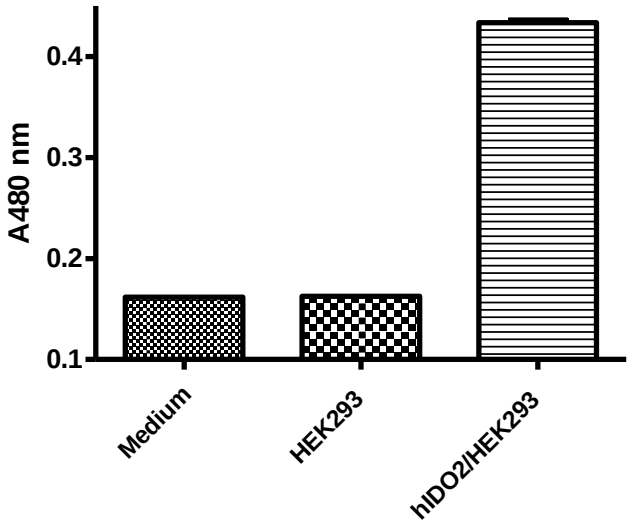

Figure 2. hIDO2 expressed in HEK293 produced Kynurenine that can be detected by absorbance at 480 nm.

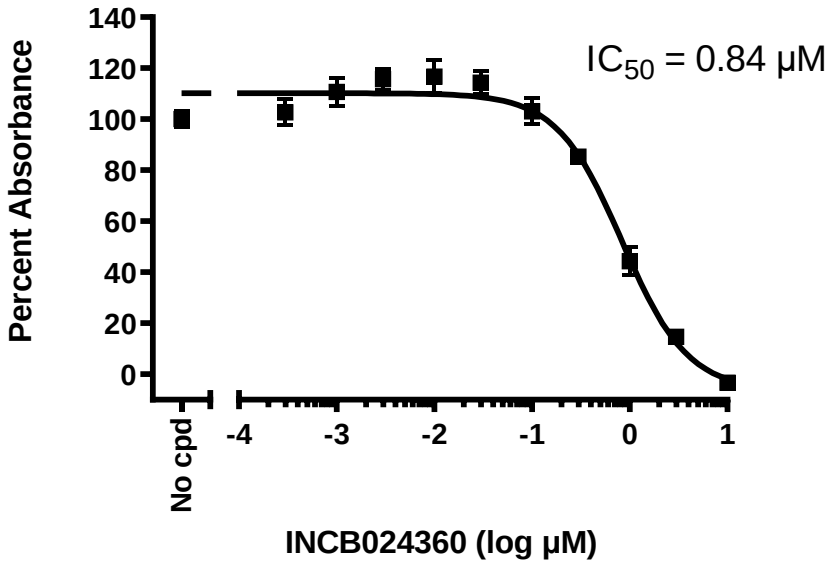

Figure 3. Dose response of hIDO2 activity in hIDO2-HEK293 cells to the reference inhibitor INCB024360. The results are shown as percentage of absorbance. The normalized absorbance for hIDO2 expressed cells without inhibitor treatment was set at 100%. The IC_{50} of INCB024360 is ~ 0.84 µM.

Sequence

N-terminal FLAG-tagged human IDO2 (accession number NM_194294, amino acids 15 to end) was cloned into a tetracycline-regulated expression vector.

Polylinker: CMV-tetracycline operator (x2)-EcoRI-FLAG-IDO2(15-end)-XhoI---SV40-neomycin^{R}

hIDO2 sequence (accession number NM_194294)

MDYKDDDDKEPHRPNVKTAVPLSLESYHISEEYGFLLPDSLKELPDHYRPWMEIANKLPQLIDAHQLQAHVDKMPLLSCQFLKGH
REQRLAHLVLSFLTMGYVWQEGEAQPAEVLPRNLALPFVEVSRNLGLPPILVHSDLVLTNWTKKDPDGFLEIGNLETIISFPGGESL
HGFILVTALVEKEAVPGIKALVQATNAILQPNQEALLQALQRLRLSIQDITKTLGQMHDYVDPDIFYAGIRIFLSGWKDNPAMPAG
LMYEGVSQEPLKYSGGSAAQSTVLHAFDEFLGIRHSKESGDFLYRMRDYMPPSHKAFIEDIHSAPSLRDYILSSGQDHLLTAYNQC
VQALAELRSYHITMVTKYLITAAAKAKHGKPNHLPGPPQALKDRGTGGTAVMSFLKSVRDKTLESILHPRG

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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*
IDO1-HEK293 Recombinant Cell line (Human)	60532	2 vials
TDO-HEK293 Recombinant Cell line (Human)	60534	2 vials
IDO2 Cellular Activity QuickDetect™ Supplements	62001-1	100 rxns.
IDO2 Cellular Activity QuickDetect™ Supplements	62001-2	1000 rxns.
IDO1 Cellular Activity QuickDetect™ Supplements	62000-1	100 rxns.
IDO1 Cellular Activity QuickDetect™ Supplements	62000-2	1000 rxns.
TDO Cellular Activity QuickDetect™ Supplements	62002-1	100 rxns.
TDO Cellular Activity QuickDetect™ Supplements	62002-2	1000 rxns.
IDO1, His-Tag	71182	50 µg
IDO2, His-tag	71194	50 µg
TDO, His-tag	71195	50 µg
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 Inhibitor Screening Assay Kit (384)	72036	384 rxns
IDO1 Fluorogenic Inhibitor Screening Assay Kit	72037	96 rxns
TDO Fluorogenic Inhibitor Screening Assay Kit	72039	96 rxns
IDO1 Cell-Based Assay Kit	72031	100 rxns
TDO Cell-Based Assay Kit	72033	100 rxns
NLG919	27337-1	10mg
INCB024360	27338-1	10mg