6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

IDO2 - HEK293 Recombinant Cell Line

Cat #: 60533

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 activators or inhibitors of IDO2 in a cellular context

Host Cell

HEK293 cells, tetracycline-inducible

Format

Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO

Storage

Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich #MP0025) to confirm the absence of Mycoplasma species.

Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) medium supplemented with 10% FBS (Life technologies #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Growth Medium 1D (BPS Cat. #79536): Thaw Medium 1 (BPS Cat. #60187) plus 600 μg/ml of Geneticin (Life Technologies #11811031), and 5 μg/ml of Blasticidin (Life Technologies # R210-01) to ensure the recombinant expression plasmid is maintained.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1D (Thaw Medium 1, Geneticin, and Blasticidin)

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no Geneticin or Blasticidin).** Spin down cells, resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin or Blasticidin)**, and transfer the resuspended cells to a T25 flask and culture in 37°C in a CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 1 **(no Geneticin or Blasticidin)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should reach ~80% confluence two days after being thawed. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 1D **(contains Geneticin and Blasticidin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add complete Growth Medium 1D and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:6 weekly.

Functional validation
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 IDO2 was confirmed by Western blotting.

IDO2 activity was confirmed by an absorbance-based assay measuring the catalyzed production of kynurenine in cell culture medium. When IDO2 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. The hIDO2 enzymatic activity in hIDO2-HEK293 cells cannot be blocked efficiently by a known hIDO1 specific inhibitor, Epacadostat, as shown by the drop in the absorbance signal.

Induction of the target protein expression
Induce cells in MEM medium, 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 0.2 µg/ml Doxycycline (MP Biomedicals #0219504401) for at least 24 hours before cell harvesting or assay.

Sample protocol to determine the effect of inhibitors on doxycycline-induced hIDO2 in hIDO2-HEK293 cells:

Materials Required but Not Supplied
- Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1D (BPS Cat. #79536)
- Doxycycline (MP Biomedicals #0219504401)
- 6.1 N Trichloroacetic acid (Sigma #T0699)

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

- Acetic acid (Sigma #320099)
- IDO2 Cellular Activity QuickDetect™ Supplements (BPS Bioscience #62001)
  Note: other formulations can be used, but significant optimization may be required.

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** using the protocol provided with the **IDO2 Cellular Activity QuickDetect™ Supplements**. Briefly, after thawing, dilute Assay Supplement 1 1:50 and Assay Supplement 2 1:100 into induction medium (0.2 µg/ml Doxycycline in Thaw Medium 1 without antibiotics).

3. Remove culture medium and treat cells with the test inhibitor in 200 µl of **Assay Medium**. Add 200 µl of **Assay Medium** containing DMSO to cell-free control wells (for determining background absorbance) and un-induced cell control wells (as an optional negative control on any basal level or leaking expression of IDO2 from hIDO2-HEK293 cells). Incubate cells at 37°C in a CO_2 incubator for 60-72 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 65°C for 15 min.

5. During the incubation, prepare **Detection Reagent Solution** by dissolving Detection Reagent (Provided in BPS Bioscience #62001) 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. Centrifuge the plate at 2500 rpm for 10 minutes to remove any sediment. If a plate centrifuge in not available, the liquid can be transferred to a microcentrifuge tube and spun briefly to pellet any solids.

7. 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.

8. 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.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Figure 1. Western Blot of the hIDO2 expressing hIDO2-HEK293 cells. Western Blot of Tet-Repressor-HEK293 parent cells (lane 1) and hIDO2-HEK293 cells (lane 2) stained with mouse anti-FLAG. 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.

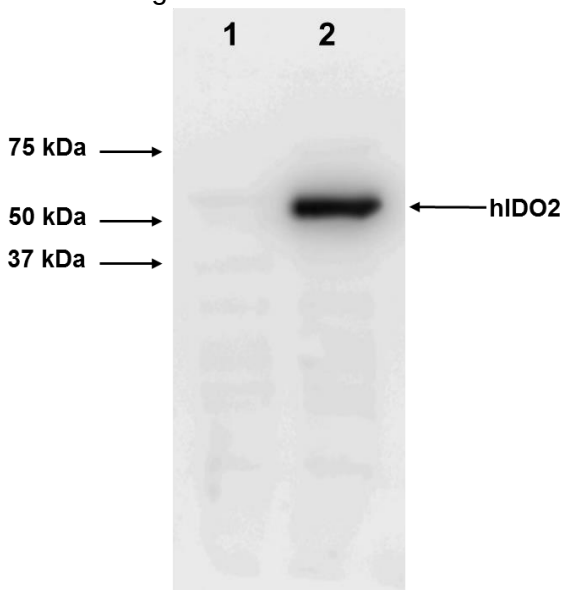
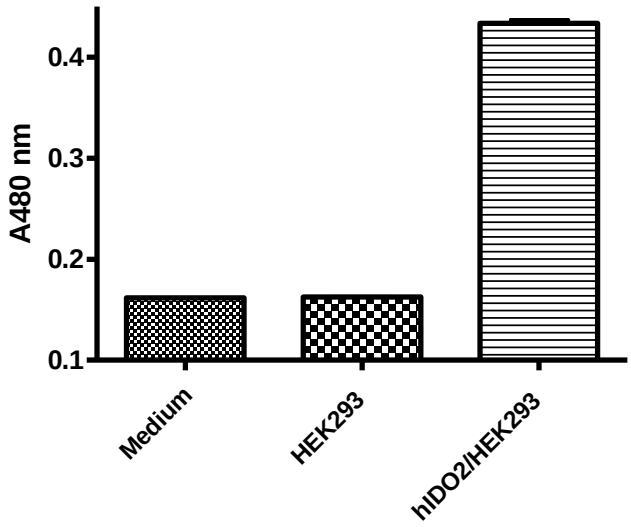


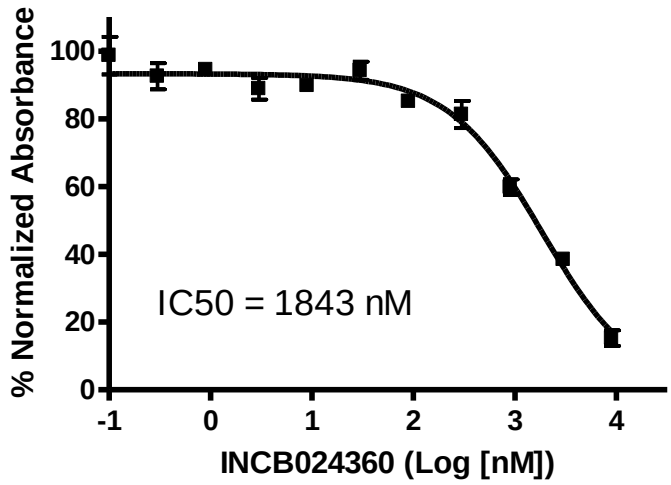
Figure 2. hIDO2 expressed in HEK293 produced Kynurenine that can be detected by absorbance at 480 nm.



The results are shown as raw absorbance data at 480 nm.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Figure 3. Dose response of hIDO2 activity in hIDO2-HEK293 cells to a hIDO1 specific 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 ~ 1843 nM

Vector and 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)

MDYKDDDDKEPHRPNVKTAVPLSLESYHISEEYGFLLPDSLKELPDHYRPWMEIANKLP
QLIDAHQLQAHVDKMPLLSCQFLKGHREQRLAHLVLSFLTMGYVWQEGEAQPAEVLP
RNLALPFVEVSRNLGLPPILVHSDLVLTNWTKKDPDGFLEIGNLETIISFPGGESLHGFIL
VTALVEKEAVPGIKALVQATNAILQPNQEALLQALQRLRLSIQDITKTLGQMHDYVDPDIF
YAGIRIFLSGWKDNPAMPAGLMYEGVSQEPLKYSGGSAAQSTVLHAFDEFLGIRHSKE
SGDFLYRMRDYMPPSHKAFIEDIHSAPSLRDYILSSGQDHLLTAYNQCVQALAELRSYHI
TMVTKYLITAAAKAKHGKPNHLPGPPQALKDRGTGGTAVMSFLKSVRDKTLESILHPRG

References
1. Metz, R., *et al.*, *Int. Immunol.* 2014; **26:** 357–367.
2. Fatokun, A., *et al., Amino Acids* 2013; **45:** 1319-1329.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

License Disclosure:
Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS Bioscience does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees; contact sales@bpsbioscience.com for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.

Related Products:

Product Name	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	10 mg
INCB024360	27338-1	10 mg