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Data Sheet

IDO1 - HEK293 Recombinant Cell Line

Cat #: 60532

Description: Stable Recombinant HEK293 cell line expressing tetracycline-inducible human indoleamine 2,3-dioxygenase (IDO1) Genbank accession number NM_002164.

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

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) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

- Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1D (BPS Cat. #79536)
- Geneticin (Sigma, #G8168) and Blasticidin S (Sigma (#15205)
- Doxycycline (MP Biomedicals #0219504401)
- PBS
- 0.05% Trypsin EDTA
- 6.1 N Trichloroacetic acid (Sigma #T0699)
- Acetic acid (Sigma #320099)
- IDO1 Cellular Activity QuickDetect™ Supplements (BPS Cat. #62000). *Note: other formulations can be used, but significant optimization may be required.*

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General Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #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.

Cells should be grown at 37°C with 5% CO_2 Growth Medium 1D. hIDO1-HEK293 cells should exhibit a typical cell division time of ~50 hours.

To thaw the cells, 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 Blastidin).** Spin down cells, resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin or Blastidin)** 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 Blastidin)** 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 Blastidin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 1D **(contains Geneticin and Blastidin)** and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:5 to 1:10 weekly or twice a week.

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 for 24 hours before cell harvesting or assay.

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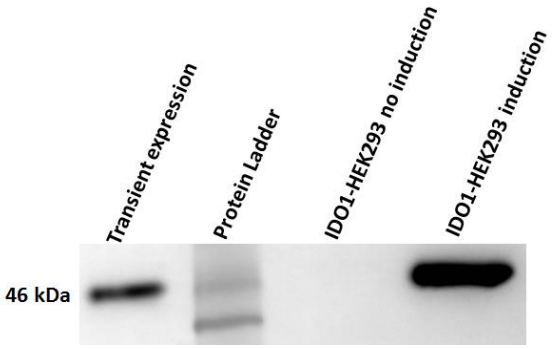
Figure 1 Western blot of hIDO1 in hIDO1-HEK293 cells.



Data shows the tetracycline-inducible expression of hIDO1 in HEK293 cells. Western blot was probed with anti-FLAG antibody (Sigma # F7425).

Functional validation
N`-terminal FLAG tagged human IDO1 has been stably integrated into HEK293 cells and its expression can be induced by tetracycline (doxycycline). The tetracycline-inducible expression of IDO1 was confirmed by Western blotting. IDO1 activity was confirmed by an absorbance-based assay measuring the catalyzed production of kynurenine in cell culture medium.

When IDO1 is expressed in hIDO1-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 hIDO1 enzymatic activity in hIDO1-HEK293 cells can be blocked by a known hIDO1 inhibitor, INCB024360, as shown by the drop in the absorbance signal to the basal level found in the un-induced HEK293 cells.

Sample protocol to determine the effect of reference inhibitor INCB024360 on doxycycline-induced hIDO1 in hIDO1-HEK293 cells:
Note: We recommend each treatment be set up in at least triplicate.

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 Blastidin)** 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) The next day (Day 1), treat the cells with 0.2 µg/ml of Doxycycline in Thaw Medium 1 without antibiotics to induce IDO1 expression.

3) On the next day (Day 2), prepare *Assay Medium* according to the protocol provided with BPS Cat. #62000. Briefly, after thawing, dilute Assay Supplement 1 1:100 and Assay Supplement 2 1:100 into cell culture medium without antibiotics.

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4) Remove culture medium and treat cells with the test inhibitor in *Assay Medium*, in this case, we used 1 µM INCB024360 in 200 µl of freshly prepared *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 IDO1 from hIDO1-HEK293 cells). Incubate cells overnight at 37°C in a CO_2 incubator. *Note: The final DMSO concentration 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. 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.

6) During the incubation, prepare *Detection Reagent Solution* by dissolving Detection Reagent (Provided in BPS Cat. #62000) 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.

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.

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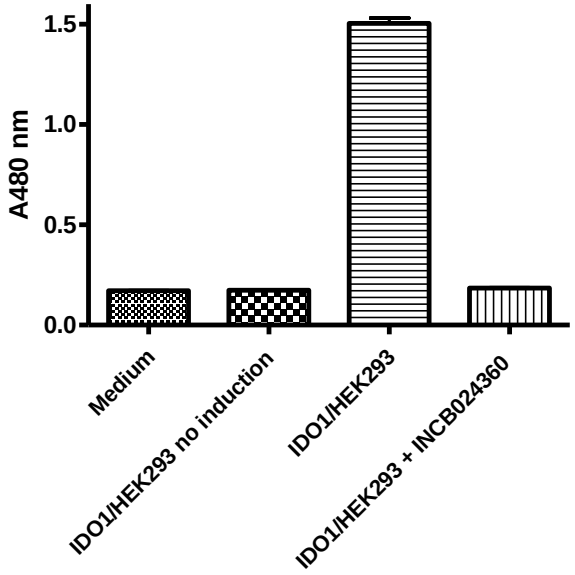
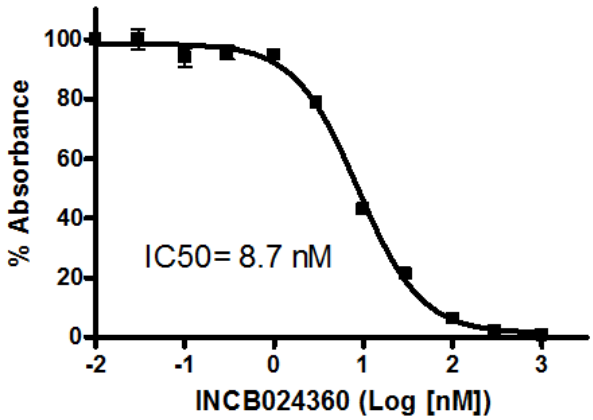
Figure 1. hIDO1-catalyzed Kyn production from L-Trp in hIDO1 – HEK293 Recombinant Cell Line (BPS Cat. #60532).



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.

Figure 2. Dose response of hIDO1 activity in hIDO1-HEK293 cells to reference inhibitor INCB024360.



The results are shown as percentage of absorbance. The normalized absorbance for hIDO1 expressed cells without inhibitor treatment was set at 100%. The IC_{50} of INCB024360 is ~ 8.7 nM.

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Vector and sequence
N-terminal FLAG-tagged human IDO1 (accession number NM_002164) was cloned into a tetracycline-regulated expression vector.

Polylinker: CMV-tetracycline operator (x 2)-EcoRI-FLAG-IDO1-XhoI---SV40-neomycin^R

hIDO1 sequence (accession number NM_002164)

MDYKDDDDKAHAMENSWTISKEYHIDEEVGFALPNPQENLPDFYNDWMFIAKHLPDLIESGQL
RERVEKLNMLSIDHLTDHKSQRLARLVLGCITMAYVWGKGHGDVRKVLPRNIAVPYCQLSKKL
ELPPILVYADCVLANWKKKDPNKPLTYENMDVLFSFRDGDCSKGFFLVSLLVEIAAASAIKVIPTV
FKAMQMQERDTLLKALLEIASCLEKALQVFHQIHDHVNPKAFFSVLRIYLSGWKGNPQLSDGLV
YEGFWEDPKEFAGGSAGQSSVFQCFDVLLGIQQTAGGGHAAQFLQDMRRYMPPAHRNFLCS
LESNPSVREFVLSKGDAGLREAYDACVKALVSLRSYHLQIVTKYILIPASQQPKENKTSEDPSKL
EAKGTGGTDLMNFLKTVRSTTEKSLLKEG

References
1. Yue, E., *et al.*, *J. Med. Chem.* 2009; **52:** 7364–7367.
2. Liu, X., *et al., Blood.* 2010; **115(17):** 3520-3530.

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