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Data Sheet

hTDO-HEK293 Recombinant Cell line

Catalog: #60534

Description: Stable recombinant HEK293 cell line expressing human tryptophan 2,3 dioxygenase (TDO2, TDO, TPH2, TRPO), Genbank accession number NM_005651.1.

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. Tryptophan 2,3 dioxygenase (TDO), is upregulated in many tumors, providing cancer cells with an avenue for immune evasion.

Applications

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

Format

Each vial contains 1.75 X 10^6 cells in 1 ml of 10% DMSO

Storage

Immediately upon receipt, store in liquid nitrogen.

Materials Required but Not Supplied

- Culture Medium: Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1B (BPS Cat. #79531)
- Geneticin
- PBS
- 0.05% Trypsin EDTA
- 6.1 N Trichloroacetic acid (Sigma #T0699)
- Acetic acid (Sigma #320099)
- TDO Cellular Activity QuickDetect™ Supplements (BPS Cat. #62002). Note: other formulations can be used, but significant optimization may be required.

Mycoplasma testing

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

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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 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) plus 400 µg/ml of Geneticin (Life Technologies #11811031) to ensure that recombinant expression is maintained. hTDO-HEK293 cells should exhibit a typical cell division time of ~33 hours.

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1B.

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).** Spin down cells, resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)**, 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)**, 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 1B **(contains Geneticin)**
.
To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add Growth Medium 1B and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:10 to 1:20 weekly or twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. Add Thaw Medium 1 and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day.

Functional validation
Human TDO has been stably expressed in HEK293 cells and its activity was characterized by an absorbance-based assay to measure the conversion of L-Trp to Kyn in cell culture medium.

When hTDO is constitutively expressed in hTDO-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 which results in production of a yellow color. The hTDO enzymatic activity in hTDO-HEK293 cells is blocked by a known hTDO inhibitor, 680C91, as shown by the drop in the absorbance signal relative to the basal level in the parental HEK293 cells.

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Sample protocol to determine the effect of reference inhibitor 680C91 on constitutively expressed hTDO in hTDO-HEK293 cells:
Note: We recommend each treatment be set up in at least triplicate.

1) On day 1, seed hTDO-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.

2) On the next day, prepare *Assay Medium* according to the protocol provided with BPS Cat. #62002. Briefly, after thawing, dilute Assay Supplement 1 1:50 and Assay Supplement 2 1:100 into cell culture medium.

3) Remove culture medium and treat cells with the test inhibitor in *Assay Medium*, in this case, we used 10 µM 680C91 in 200 µl of freshly prepared *Assay Medium*. Add 200 µl of Culture Medium containing DMSO to cell-free control wells (for determining background absorbance) and parental HEK293 cell control wells, if desired (as a negative control for any basal level of TDO from HEK293 cells). Incubate cells overnight at 37°C in a CO_2 incubator. *Note: The final DMSO concentration should not exceed 0.3%.*

4) On day 3, remove 140 µl of medium from each well of the cell culture and transfer into a new 96-well plate. Add 10 µl of 6.1 N trichloroacetic acid to each well. Incubate the plate at 50°C for 30 minutes. 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.

5) During the incubation, prepare *Detection Reagent Solution* by dissolving Detection Reagent (provided with BPS Cat. #62002) 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) 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.

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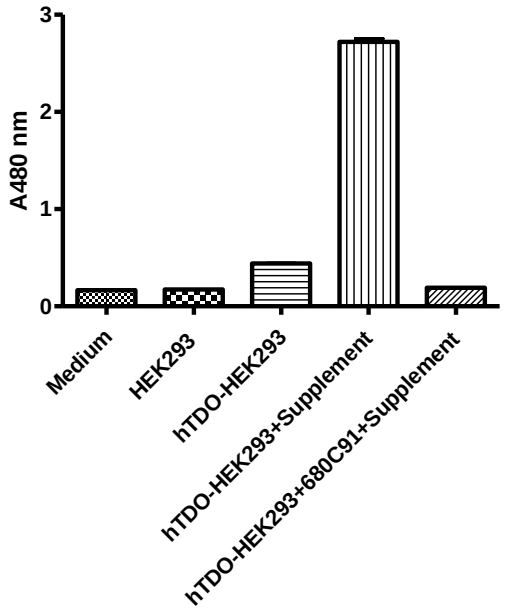
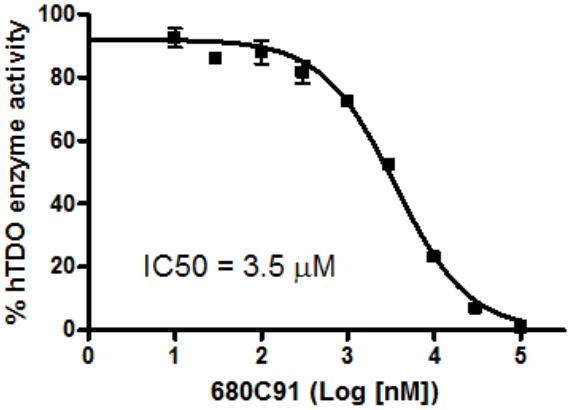
Figure 1. hTDO-catalyzed Kyn production from L-Trp in hTDO-HEK293 Recombinant Cell Line (BPS Cat. #60534).



680C91 completely blocks hTDO enzyme activity at a concentration of 100 µM. The results are shown as raw absorbance data at 480 nm. Conditions from left to right: medium only (no cells), parental HEK293 cells plus all assay components, hTDO-HEK293 plus growth medium (no assay components), hTDO-HEK293 plus all assay components, and hTDO-HEK293 plus all assay components and 100 µM 680C91.

Figure 2. Dose response of hTDO activity to reference inhibitor 680C91 in hTDO-HEK293 cells.



The results are shown as percentage of absorbance. The normalized absorbance for hTDO transfected cells without inhibitor treatment was set at 100%. The IC_{50} of 680C91 is ~ 3.5 µM

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Vector and sequence
N-terminal FLAG-tagged human TDO (accession number NM_005651) was cloned into pcDNA3.1 vector (LifeTechnology).

Polylinker: CMV-HindIII-KpnI-BamHI-FLAG-**TDO**-XhoI-XbaI-ApaI------SV40-neomycin^{R}

Flag-hTDO sequence (accession number NM_005651)

MDYKDDDDKSGCPFLGNNFGYTFKKLPVEGSEEDKSQTGVNRASKGGLIYGNYLHLEKVLNA
QELQSETKGNKIHDEHLFIITHQAYELWFKQILWELDSVREIFQNGHVRDERNMLKVVSRMHRV
SVILKLLVQQFSILETMTALDFNDFREYLSPASGFQSLQFRLLENKIGVLQNMRVPYNRRHYRD
NFKGEENELLLKSEQEKTLLELVEAWLERTPGLEPHGFNFWGKLEKNITRGLEEEFIRIQAKEES
EEKEEQVAEFQKQKEVLLSLFDEKRHEHLLSKGERRLSYRALQGALMIYFYREEPRFQVPFQLL
TSLMDIDSLMTKWRYNHVCMVHRMLGSKAGTGGSSGYHYLRSTVSDRYKVFVDLFNLSTYLIP
RHWIPKMNPTIHKFLYTAEYCDSSYFSSDESD

References
1. Liu, X., *et al., Blood.* 2010; **115(17):** 3520-3530.

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