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Data Sheet

IDO2 Cellular Activity QuickDetect™ Supplements
Catalog: #62001-2

Description: The IDO2 Cellular Activity QuickDetect™ Supplements are designed to complement the hIDO2-HEK293 Recombinant Cell Line (BPS Cat. #60532) or other IDO2-expressing cell lines. This kit contains the cell culture medium supplements necessary for activation of IDO2 and for the analysis and detection of Indoleamine 2,3 dioxygenase 2 (IDO2)-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-tryptophan (L-Trp) is an essential amino acid necessary for protein synthesis in mammalian cells. 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. Additionally, the released degradation products of L-Trp have immunosuppressive functions. Indoleamine 2,3-dioxygenases (IDO1 & IDO2), two of the rate limiting enzymes in this pathway, are upregulated in many tumors and provide cancer cells with an avenue for immune evasion.

Applications

- Monitor IDO2 pathway activity
- Screen for activators or inhibitors of IDO2 in a cellular context

Format

Component	Amount	Storage
IDO2 Assay Medium Supplement 1	2 x 1 ml	4°C
IDO2 Assay Medium Supplement 2	4 x 500 ml	-20°C
Detection Reagent	2 g	Room Temp.

Materials Required but Not Supplied

hIDO2-HEK293 Recombinant Cell Line (BPS, Cat. #60533) or other IDO2-expressing cell line and appropriate cell culture medium
1-Methyl-D-Tryptophan (Sigma, #452483) or 1-Methyl-L-Tryptophan (Sigma #447439)
6.1 N (concentrated) trichloroacetic acid*
17.4 N (concentrated) glacial acetic acid*
**Note: both trichloroacetic acid and acetic acid are strongly corrosive acids; please use gloves and appropriate protective clothing.*

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General Assay Procedure

1) On day 1, seed cells at a density of 30,000 cells in 100 µl of growth medium *without antibiotic* into each well of a tissue culture-treated 96-well plate. Incubate cells at 37°C in a CO_2 incubator overnight.

2) Prepare fresh *Assay Medium* by diluting **IDO2 Assay Medium Supplement 1** 1:100 and **IDO2 Assay Medium Supplement 2** 1:100 into cell culture medium.

3) Dilute the IDO2 inhibitor 1-Methyl-D-Tryptophan (10 µM) or test compound to the desired concentration in fresh *Assay Medium*. Remove the cell culture medium from transfected cells and replace with 200 µl of *Assay Medium* containing the inhibitor compound. 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%.*

4) On the next day, 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.

5) Prepare *Detection Reagent Solution* by dissolving **Detection Reagent** 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 fresh *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: 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.

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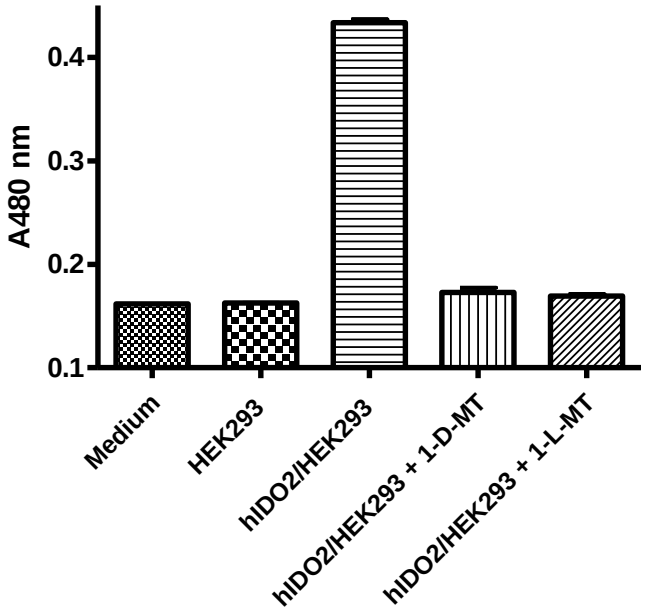
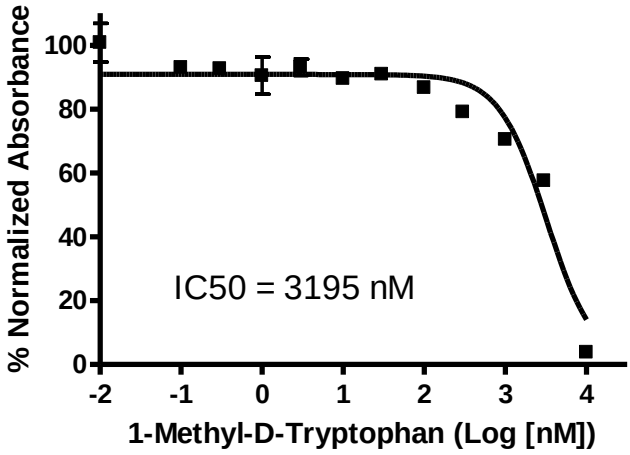
Figure 1. hIDO2 expressed in HEK293 produced Kynurenine from L-Trp and hIDO2 enzyme activity was inhibited by known IDO2 inhibitors, 1-Methyl-D-tryptophan and 1-Methyl-L-tryptophan.



1-Methyl-D-Tryptophan and 1-Methyl-L-Tryptophan completely block hIDO2 enzyme activity at a concentration of 10 µM. The results are shown as raw absorbance data at 480 nm.

Figure 2. Dose response of hIDO2 activity in hIDO2-HEK293 cells to reference inhibitor 1-Methyl-D-tryptophan.



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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 1-Methyl-D-tryptophan is ~ 3.2 µM

References

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

Related Products

Product Name	Catalog #	Size
hIDO2-HEK293 Recombinant Cell Line	60533	2 vials
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