Aurora Biolabs
High Purity Protein for Research

IDO1 Activity Assay Kit for Inhibitor Screening

Catalog Number: 910010

Background

Tryptophan is an essential amino acid that is involved in protein synthesis and regulation of the local immune response by T lymphocytes. Indoleamine 2,3-dioxygenase-1 (IDO1) catalyzes oxidation of tryptophan to N-formylkynurenine (NFK), the initial and rate limiting step in the pathway of catabolism of tryptophan. Over expression of IDO1 in variety of cancers results in the depletion of Tryptophan and the accumulation of kynurenine, that have been proposed as mechanisms that contribute to the suppression of the immune response.

Assay Principle

IDO1 Activity Assay Kit is designed to measure the activity of IDO1 enzyme and can be used for screening IDO1 inhibitors. The activity assay is carried out on a 96-well plate. After incubation of the enzyme, substrate and inhibitors, absorbance of the product NFK is measure at 321nm, and IDO1 activity is calculated based on the absorbance value. The kit contains enough solutions for 100 reactions.

Application

High throughput screening of IDO1 inhibitors.

Plate Reader

Spectrophotometer capable of measuring absorbance at 321 nm.

Components

Catalog number	Item	Amount	Storage
910013	2x Assay Buffer	25 mL	-20°C
910011	Recombinant IDO1 protein	160 µL	-80°C
910012	40 mM L-Tryptophan	200 µL	-80°C
	96-well microplate	1	Room temperature

Materials needed but not supplied

1. Microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

Assay protocol

1. Prepare 1X assay buffer containing 1 mM DTT (1X DTT-containing assay buffer)

   For example, mix 998 µl distilled water with 1000 µl of 2X assay Buffer (Catalogue number: 910013) and 4 µl of 0.5 M DTT. Make only enough 1X DTT-containing assay buffer as needed for the assay. Store the remaining 2X assay buffer at -20°C.

2. Prepare the inhibitor compound solution

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 µl to the 20 µl reaction).

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare a series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

3. Prepare IDO1 protein solution

   Thaw IDO1 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C. Note: IDO1 protein is sensitive to freeze/thaw cycles. Limit the number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the IDO1 protein 50-fold (for example: 10 µL IDO1 + 490 µL 1X DTT-containing assay buffer).

   Add 80 µl of diluted protein solution to each of positive control well and inhibitor test wells.

   Add 80 µl of 1X DTT containing buffer to each of the negative control wells.

4. Add inhibitor solution

   Add 20 µl of diluted inhibitor solution to each inhibitor test wells.

   Add 20 µl of 1X DTT-containing assay buffer to each positive and negative control wells.

5. Prepare Substrate solution

   Dilute Tryptophan solution 50-fold (for example: 10 µL Tryptophan + 490 µL 1X DTT-containing assay buffer).

   Add 100 µl of diluted substrate solution to each well.

6. Incubate the reaction at 30°C for 2 hours.

7. Measure absorbance

Measure absorbance at 321 nm in a plate reader.

Protocol Summary

Component	Negative Control	Positive Control	Test inhibitor
1X DTT-containing assay buffer	100 µl	20 µl	-
Diluted IDO1 solution	-	80 µl	80 µl
Diluted inhibitor solution	-	-	20 µl
Diluted Tryptophan	100 µl	100 µl	100 µl
Total Volume	200 µl	200 µl	200 µl

Incubate at 30°C for 2 hours.

Data Analysis

1. Calculate percentage activity

   In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\% \text{ Activity} = \frac{S - N}{\mathrm{P} - \mathrm{N}} X100$$

Data Presentation