CREATIVE BIOMART®
Assay Kit

IDO1 Inhibitor Mechanism of Action Assay Kit

Product Information

Cat.No.

Kit-1763

Product Overview

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. Inhibitors of IDO1 can bind either irreversibly (covalent binding to the protein) or reversibly (non-covalent association) to the enzyme. The IDO1 Inhibitor Mechanism of Action Assay Kit allows researchers to determine the mechanism of IDO1 inhibitor binding. At high concentrations, e.g., 10x the IC50 concentration of the inhibitor, both reversible and irreversible inhibitors will interfere with IDO1 activity. However, after sufficient dilution, e.g. to 0.3x the IC50 concentration, reversible inhibitors will dissociate, relieving inhibition of the IDO1 enzyme. Irreversible inhibitors will not dissociate, and will continue to inhibit the IDO1 enzyme at the same level. Therefore, by monitoring enzymatic activity after inhibitor dilution, the mechanism of binding to the IDO1 protein can be determined.

Size

50 reactions

Description

The IDO1 Inhibitor Mechanism of Action (MoA) Assay Kit is designed to determine the mechanism of IDO1 enzyme inhibition (e.g., reversible or irreversible inhibition). The IDO1 Mechanism of Action Assay Kit is simple to use. Inhibitor and enzyme are preincubated allowing the inhibitor to bind. After preincubation, the mixture is diluted into reaction buffer containing the L-tryptophan (L-Trp) substrate and all necessary coupled reaction components. This allows reversible inhibitors to dissociate, while irreversible inhibitors remain associated with the IDO1 enzyme. IDO1 activity is determined by measuring the absorption of the reaction product at λ=320-325 nm.

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Applications

Useful for the study of IDO1 enzymology, screening inhibitors, and selectivity profiling.

Storage

Stable at least 6 months from date of receipt, when stored as directed. Kit components require different storage conditions. Be sure to store each component at the proper temperature upon arrival.

Kit Components

IDO1 His-Tag: 40 µg; -80°C IDO1 Reaction Solution: 10 ml; -80°C 1x IDO1 Assay Buffer: 25 ml; -80°C Reversible Reference Inhibitor (1000x): 50 µl; -20°C Irreversible Reference Inhibitor (1000x): 50 µl; -20°C UV transparent 96-well Reaction Plate: 1; Room Temp. 96-well Preincubation Plate: 1; Room Temp.

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