CREATIVE BIOMART®
Assay Kit

IKK-alpha/beta Kinase (Human) Assay/Inhibitor Screening Assay Kit

Product Information

Cat.No.

Kit-0475

Product Overview

IKK-alpha/beta Kinase (Human) Assay/Inhibitor Screening Assay Kit is a single-site, non-quantitative immunoassay for activities of IKK-alpha and IKK-beta. Plates are pre-coated with a substrate corresponding to recombinant IkappaB-alpha, which contains two serine residues that are phosphorylated by IKK-alpha and IKK-beta (IkappaB kinases). The detector antibody specifically detects only the phosphorylated form of IkappaB-alpha.

Description

Although a number of recent studies suggest that IκB degradation and nuclear translocation of NF-κB may not be the sole regulatory events in the transcription of NF-κB-dependent genes, it has been proposed that there is the central dogma of NF-κB activation, which suggests that NF-κB is sequestered in the cytoplasm in resting cells by the inhibitory IκB proteins. In response to a variety of agonists, IκB is rapidly phosphorylated, ubiquitinated, and degraded, thus releasing NF-κB for translocation into the nucleus to initiate gene transcription. IκB kinase (IKK) is the convergence point in most signaling pathways activated by many stimuli leading to the inducible phosphorylation and degradation of IκB. IKK is a multisubunit complex that contains two catalytic subunits, IKKa and IKKβ, and the regulatory subunit IKKγ. Gene knock out studies have clearly demonstrated that IKKβ and IKKγ subunits of the IKK complex are required for NF-κB activation by all known pro-inflammatory stimuli including lipopolysaccharide (LPS), TNF, and IL-1. Thus a selective inhibitor of IKKβ would not only be of great interest as a potential anti-inflammatory agent but also as a valuable tool to understand the mechanisms regulating NF-κB activation by these inflammatory agonists.

Applications

1) Screening inhibitors or activators of IKKa and IKKβ.2) Detecting the effects of pharmacological agents on IKKa and IKKβ activity.

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Target Species

Human

Usage

For research use only (RUO)

Storage

• Upon receipt store the ATP at -20°C• Upon receipt store all other components at 4°C; Do not expose reagents to excessive light

Kit Components

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with recombinant IκBa as substrate of IKK.10X Wash Buffer: One 100 mL bottle of 10X buffer containing 2%Tween -20Kinase Buffer: One bottle containing 20 mL of 1X buffer; used for Kinase Reaction Buffer and sample dilution.20X ATP: Lyophilized ATP Na2 salt. Reconstitute contents of vial with 1.6 mL of H2O. Mix gently until dissolved. Final concentration of ATP should be 1.25 mM ATP. The ATP solution can be stored in small aliquots (e.g. 100 µL) at -20°C. The 1 mM ATP stock solution must be diluted to 62.5 µM in Kinase Reaction Buffer at the time of the assay.Anti-Phospho-IκBa S32 Monoclonal Antibody (AS-2E8): One vial containing 12 mL of anti-phospho-IκBa S32 monoclonal antibody (AS-2E8). Ready to use.HRP conjugated Anti-mouse IgG: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-mouse IgG. Ready to use.Substrate Reagent: 12 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.Stop Solution: One bottle supplied ready to use, containing 12 mL of 0.5 N H2SO4.

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