CREATIVE BIOMART®
Assay Kit

p56Lck Kinase (Human) Assay/Inhibitor Screening Assay Kit

Product Information

Cat.No.

Kit-0508

Product Overview

p56Lck Kinase (Human) Assay/Inhibitor Screening Assay Kit is a single-site, non-quantitative immunoassay for kinase activity of recombinant catalytic domain of Lck. Plates are pre-coated with a newly designed "Tyrosine kinase-binding module-1", which can easily bind recombinant catalytic domain of Lck, subsequently activate Lck kinase activity on a microtiter plate. The detector antibody is PY-39, an antibody that specifically detects the phosphotyrosine residue on recombinant catalytic domain of Lck itself, which means that this kit measures the intensity of autophosphorylation of Lck catalytic domain.

Description

Lck is a 56-kDa protein tyrosine kinase that is predominantly expressed in T lymphocytes. A member of the Src kinase family, it has a unique N-terminal region followed by SH3, SH2, and catalytic domains. Lck is an important protein tyrosine kinase in lymphocytes; its overexpression renders T cells hypersensitive to antigen stimulation, and an Lck-deficient T cell line, J.CaM1, exhibits dramatically reduced protein tyrosine phosphorylation following T cell receptor (TCR) cross-linking. Furthermore, genetic experiments have shown that mice deficient in Lck or expressing a dominant-negative mutant form of Lck exhibit a severe defect in T cell maturation. Lck is localized to the membrane through myristylation and palmitylation and a portion of cellular Lck associates with the cytoplasmic tail of CD4 via cysteine residues. CD4 binds to class II major histocompatibility complex molecules on antigen-presenting cells, and this interaction between CD4 and major histocompatibility complex activates Lck, perhaps through conformational changes. The Lck associated with CD4 propagates key biochemical signals in CD4 co-receptor function. Like all Src family kinases, Lck is activated and inhibited by tyrosine phosphorylation, Tyr-394 is the site of stimulatory phosphorylation, whereas Tyr-505 is the site of inhibitory phosphorylation.

Applications

1) Screening inhibitors or activators of recombinant catalytic domain of Lck.2) Detecting the effects

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of pharmacological agents on recombinant catalytic domain of Lck.

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 "Tyrosine kinase-binding module-1".10X Wash Buffer: One 100 mL bottle of 10X buffer containing 2%Tween -20.Kinase Buffer: One 20 mL bottle of 1X buffer used for Kinase Reaction Buffer and sampledilution.20X ATP: Lyophilized ATP Na2 salt. Reconstitute contents of vial with 2 mL of H2O. Mix gently until dissolved. Final concentration of ATP should be 1 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 50 µM in Kinase Reaction Buffer at the time of the assay.HRP conjugated Detection Antibody: One bottle containing 12 mL of HRP (horseradish peroxidase) conjugated anti-phosphotyrosine monoclonal antibody (PY-39).Substrate Reagent: One bottle containing 12 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.Stop Solution: One bottle supplied ready to use, containing 12 mL of 1.25 N H2SO4. Ready to use.

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