

Polo-like kinase 1 (Human) Assay/Inhibitor Screening Assay Kit

Product Information

Cat.No.

Kit-0705

Product Overview

Polo-like kinase 1 (Human) Assay/Inhibitor Screening Assay Kit is a single-site, non-quantitative immunoassay for Plk1 activity. Plates are pre-coated with a substrate corresponding to recombinant Protein-X, which contains threonine residues that can be efficiently phosphorylated by Plk1. The detector antibody specifically detects only the phosphorylated form of threonine residue on Protein-X.

Description

Polo-like kinases (Plk) have been shown to be important contributors to several cell-cycle events. Genetic and biochemical experiments in various organisms indicate that polo-like kinases regulate diverse cellular events at multiple mitotic stages. Genetic studies in *Drosophila* and yeast indicate plks function in centrosome assembly and separation during the formation of the bipolar spindle. *Drosophila* polo mutants reveal phenotypes of hyper-condensed chromosomes, monopolar spindles, disorganized spindle poles, and abnormal chromosome segregation. *Schizosaccharomyces pombe* plo1 displays similar phenotypes, such as the formation of monopolar spindles or a failure in septum formation after nuclear division. The budding yeast polo-like kinase homolog, Cdc5, seems to play an important role in actin ring formation and cytokinesis. In mammalian cells, antibody microinjection suggests a role for Plk1 in centrosome maturation. Mammalian Plk1 was further shown to phosphorylate specifically at least three components of APC, and to activate APC to ubiquitinate cyclin B in an in vitro-reconstituted system. More recent studies demonstrated that polo kinase activity plays a pivotal role in the separation of sister chromatids during mitosis.

Applications

1) Screening inhibitors or activators of Plk1. 2) Detecting the effects of pharmacological agents on Plk1 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 Protein-X as Plk1 substrate.

10X Wash Buffer: One 100 mL bottle of 10X buffer containing 2% Tween-20.

Kinase 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 1.25 mM ATP stock solution must be diluted to 62.5 µM in Kinase Reaction Buffer at the time of the assay.

Anti-Phospho-Threonine Polyclonal Antibody (PPT-07): One vial containing 12 mL of anti-phospho-threonine polyclonal antibody (PPT-07). Ready to use.

HRP conjugated Anti-rabbit IgG: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-rabbit IgG. Ready to use.

Substrate Reagent: 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle supplied ready to use, containing 20 mL of 1 N H₂SO₄.