PLK1, Active
Full-length recombinant protein expressed in Sf9 cells

Catalog # P41-10H
Lot # A1376-2

Product Description
Recombinant full-length human PLK1 was expressed using baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_005030.

Gene Aliases
STPK13

Formulation
Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol.

Storage and Stability
Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background
PLK1 is a member of the Polo-Like Kinase family that localizes to centrosomes or spindle pole bodies and undergoes dramatic subcellular relocation during the cell cycle. Deregulated activities of PLKs often result in abnormalities in centrosome duplication, maturation, and/or microtubule dynamics (1). PLKs also regulate the function of the Golgi complex. Deregulated expression of human PLK1 is strongly correlated with the development of many types of malignancies, and ectopic expression of PLK1 dominant negative protein leads to rapid cell death (2).

References

Specific Activity
The specific activity of PLK1 was determined to be 20 nmol/min/mg as per activity assay protocol.

Purity
The purity of PLK1 was determined to be >95% by densitometry, approx. MW 70kDa.

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Activity Assay Protocol

Reaction Components

**Active Kinase** (Catalog #: P41-10H)
Active PLK1 (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PLK1 for optimal results).

**Kinase Dilution Buffer III** (Catalog #: K23-09)
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

**Kinase Assay Buffer I** (Catalog #: K01-09)
Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl2, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[33P]-ATP Assay Cocktail**
Prepare 250µM [33P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [33P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at –20°C.

**10mM ATP Stock Solution** (Catalog #: A50-09)
Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

**Substrate** (Catalog #: C03-54BN)
Casein, Dephosphorylated, diluted in distilled H2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [33P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active PLK1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
   - Component 1. 10µl of diluted Active PLK1 (Catalog #P41-10H)
   - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #C03-54BN)
   - Component 3. 5µl distilled H2O (4°C)
Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H2O.
Step 5. Initiate the reaction by the addition of 5 µl [33P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P33]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5 µl [33P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected cpm from reaction / [(SA of 33P-ATP in cpm/pmol)*[Reaction time in min]*([Enzyme amount in µg or mg]*[(Reaction Volume) / (Spot Volume)]]

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