PKN2/PRK2, Active
Recombinant protein expressed in Sf9 cells

Catalog # P71-10G
Lot # H032-1

Product Description
Recombinant full-length human PKN2/PRK2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_006256.

GeneAliases
PRKCL2, PKN2, PRK2, PAK2, Pak-2, PRO2042, MGC71074, MGC130606

Formulation
Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 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
PKN2 (also known as PRK2) is a Rho effector and a member of the protein kinase C superfamily of serine/threonine kinases. PKN2 is an essential regulator of both entry into mitosis and exit from cytokinesis in HeLa S3 cells (1). PKN2 is required for abscission of the midbody at the end of the cell division cycle and for phosphorylation and activation of Cdc25B, the phosphatase required for activation of mitotic cyclin/Cdk1 complexes at the G2/M transition. C-terminus of PKN2 could be a potential drug target for effector-specific pharmacological intervention of Rho-mediated biological processes (2).

References

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Specific Activity
The specific activity of PKN2/PRK2 was determined to be 74 nmol/min/mg as per activity assay protocol.

Purity
The purity of PKN2/PRK2 was determined to be >75% by densitometry, approx. MW 145 kDa.

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Specific Activity
74 nmol/min/mg
Specific Lot Number
H032-1

Storage & Shipping
-75% ->75%
0.1 µg/µl
1yr at −70°C from date of shipment
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. Product shipped on dry ice.
Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P71-10G)
Active PKN2/PRK2 (0.1 µg/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 PKN2/PRK2 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 ß-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

Substrate (Catalog #: C50-58)
CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in 25mM Tris-HCl buffer (pH 7.5) to a final concentration of 1mg/ml.

[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 I (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.

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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