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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
W04-11G -05	5 µg
W04-11G -10	10 µg

WNK3, Active

Recombinant human protein expressed in Sf9 cells

Catalog # W04-11G

Lot # H2931-12

Product Description

Recombinant human WNK3 (1-571) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_001002838.

Gene Aliases

PRKWNK3; FLJ30437; FLJ42662; KIAA1566

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 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.

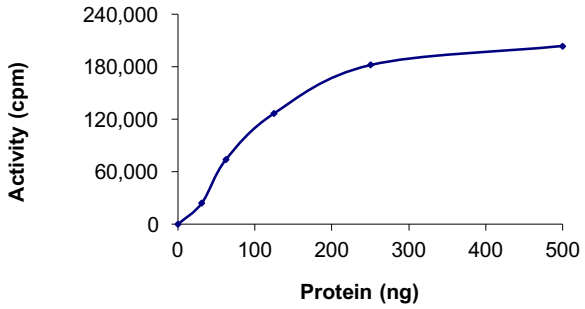
Scientific Background

WNK3 is a member of serine-threonine protein kinases, which was named the with-no-lysine family (1). WNK3 is expressed in several tissues including kidney and brain (1). WNK3 can regulate the activity of the electroneutral cation-coupled chloride cotransporters by activating NKCC1/2 and NCC and inhibiting KCCs (1). WNK3 kinase function is required for normal blood pressure homeostasis and the control of neuronal excitability (2). Also, overexpression of WNK3 increases the survival of Hela cells through caspase-3 pathway (3).

References

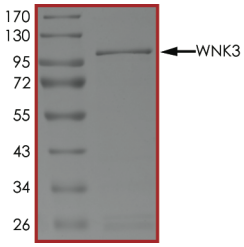
1. Rinehart J., et.al: WNK3 kinase is a positive regulator of NKCC2 and NCC, renal cation-Cl-cotransporters required for normal blood pressure homeostasis. Proc Natl Acad Sci USA.; 102(46):16777-82, 2005.
2. Kahle KT., et.al: WNK3 modulates transport of Cl- in and out of cells: implications for control of cell volume and neuronal excitability. Proc Natl Acad Sci USA.; 102(46):16783-8, 2005.
3. Veríssimo F., et.al: Protein kinase WNK3 increases cell survival in a caspase-3-dependent pathway. Oncogene, 25 (30): 4172-82, 2006.

Specific Activity



The specific activity of WNK3 was determined to be **23.2 nmol/min/mg** as per activity assay protocol.

Purity



The purity of WNK3 was determined to be **>90%** by densitometry, approx. MW **91kDa**.

WNK3, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	W04-11G
Specific Activity	23.2 nmol/min/mg
Lot #	H2931-12
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: W04-11G)

Active WNK3 (0.1μg/μl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 WNK3 for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 12.5mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). 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 II (Catalog #: K02-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: M42-51N)

Myelin Basic Protein (MBP) substrate diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active WNK3, 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 WNK3 (Catalog #W04-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
- **Component 3.** 5μl of distilled H_2O

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 H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-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 H_2O) 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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