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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
W03-11G -05	5 µg
W03-11G -10	10 µg

WNK2, Active

Recombinant human protein expressed in Sf9 cells

Catalog # W03-11G
Lot # K1792-4

Product Description

Recombinant human WNK2 (1-489) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_006648.

Gene Aliases

KIAA1760; NY-CO-43; P/OKcl.13; PRKWNK2; SDCCAG43

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.

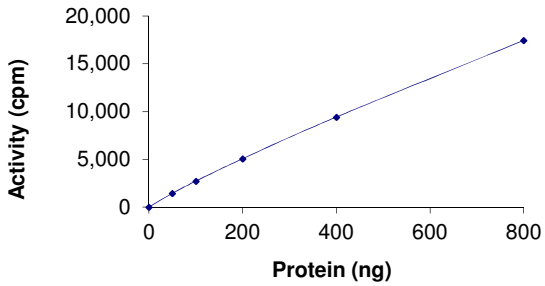
Scientific Background

WNK2 is a serine/threonine kinase of the WNK (with no lysine (K)) protein kinase subfamily that also include WNK1, 3 and 4. The WNK kinases feature a cysteine residue in place of a lysine residue conserved in subdomain II of most kinases. WNK2 is predominantly expressed in brain, heart muscle, small intestine, and colon. As a tumor suppressor, WNK2 is involved in the modulation of growth factor-induced cancer cell proliferation through the MEK1/ERK1/2 pathway. WNK2 expression was found down-regulated epigenetically by promoter methylation in human gliomas.

References

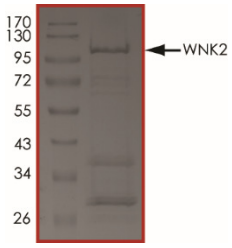
1. Veríssimo F, Jordan P. WNK kinases, a novel protein kinase subfamily in multi-cellular organisms. Oncogene. 2001 Sep 6;20(39):5562-9.
2. Moniz S. et al. Protein kinase WNK2 inhibits cell proliferation by negatively modulating the activation of MEK1/ERK1/2. Oncogene. 2007 Sep 6;26(41):6071-81.
3. Moniz S, et al. Loss of WNK2 expression by promoter gene methylation occurs in adult gliomas and triggers Rac1-mediated tumour cell invasiveness. Hum Mol Genet. 2013;22:84–95.

Specific Activity



The specific activity of WNK2 was determined to be **2.0 nmol/min/mg** as per activity assay protocol.

Purity



The purity of WNK2 was determined to be **>70%** by densitometry, approx. MW **100kDa**.

WNK2, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	W03-11G
Specific Activity	2.0 nmol/min/mg
Specific Lot #	K1792-4
Purity	>70%
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 #: W03-11G)

Active WNK2 (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 WNK2 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 $MgC1_2$, 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 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.

Substrate (Catalog #: C01-58)

PKA-sub synthetic peptide substrate (CGRTGRRNSI-amide) was 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 WNK2, 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 WNK2 (Catalog #W03-11G)
- **Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #C01-58)
- **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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