

Certificate of Analysis

WNK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-658, 14-658-K, 14-658M

Parent Lot # D8JN021U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, human WNK3, amino acids 1–434. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 53.1kDa.

Specific Activity (Parent lot# D8JN021U): 61U/mg, where one unit of WNK3 activity is defined as 1nmol phosphate incorporated into 0.333mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3.36mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

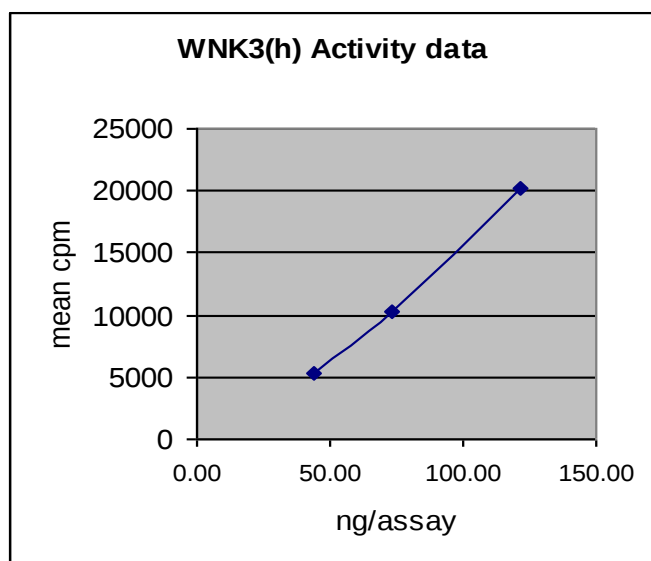
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

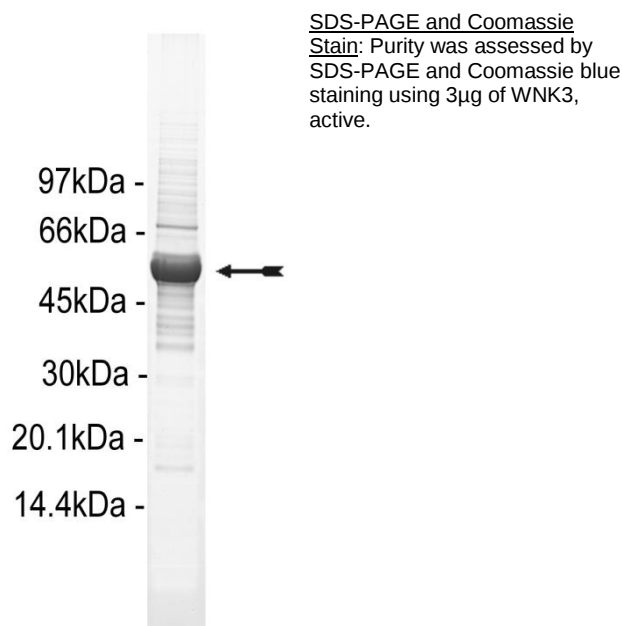
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 44–121ng of this lot of enzyme phosphorylated 0.333 mg/ml myelin basic protein (MBP) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as WNK3 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **Myelin Basic Protein (MBP):** Use at a final concentration of 0.333mg/ml. Make a 3.33mg/ml stock. Add 2.5µl stock per assay point.
3. **WNK3, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 44–121ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **myelin basic protein (MBP)**.
3. Add **2.5µl (44–121ng) WNK3, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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WNK3 Sequence Information

Protein	Human WNK3
Tags	N-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of human WNK3
Accession number	GenBank NM_020922

Recombinant WNK3 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MATDSGDPAS TEDSEKPDGI SFENRVPQVA
61 ATLTVEARLK EKNSTFSASG ETVERKRFFR KSVEMTEDDK VAESSPKDER IKAAMNIPRV
121 DKLPSNVLRG GQEVKYEQCS KSTSEISKDC FKEKNEKEME EEAEMKAVAT SPSGRFLKFD
181 IELGRGAFKT VYKGLDTEW VEWAWCELQD RKLTKAEQQR FKEEAEMLK LQHPNIVRFY
241 DSWESILK GK CIVLVTELM TSGTLKTYLK RFKVMKPKVL RSWCRQILKG LQFLHTRTPP
301 IIHRDLKCDN IFITGPTGSV KIGDLGLATL MRTSFAKSVI GTPEFMAPEM YEEHYDESVD
361 VYAFGMCMLE MATSEYPYSE CQNAAQIYRK VTSGIKPASF NKVTDPEVKE IIEGCIRQNK
421 SERLSIRDLL NHAFFAEDTG LRVELAEEDD CSNSSLALRL WVED

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Recombinant WNK3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atggccactg attcagggga tccagccagc
121 acagaagatt ctgagaaacc tgatggaatt tcatttgaaa acagagttcc ccaggctcgt
181 gcaactttga cagtagaagc tagactaaag gagaaaaaca gtaccttctc tgcttctggg
241 gaaactgtag aaaggaagag atttttccga aagagtgttg aaatgacgga agatgacaaa
301 gttgccgaat catcccccac agatgagaga attaaggctg caatgaatat tccaagagta
361 gataagcttc cttcaaatgt gttgagaggt ggacaagaag ttaaatatga acagtgttca
421 aagtcaacct cagaaatctc aaaagattgt ttcaaggaga aaaatgaaaa ggaaatggaa
481 gaagaagcag aaatgaaggc tgtagctact tctcctagtg gcagattcct gaaatttgac
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1201 aataaagtca ctgatcctga agtcaaagaa atcattgaag gatgtattcg tcaaaacaaa
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1321 ctgagggtgg agttagcaga agaagatgat tgctcaaatt catcccttgc ttttaagactc
1381 tgggttgaag actaa

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