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Certificate of Analysis

WNK2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-678, 14-678-K, 14-678M
Parent Lot # D7BN052U

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 WNK2, amino acids 166–489, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 84% by SDS-PAGE and Coomassie blue staining. MW = 41.2kDa.

Specific Activity (Parent lot# D7BN052U): 37U/mg, where one unit of WNK2, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.16mg/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.

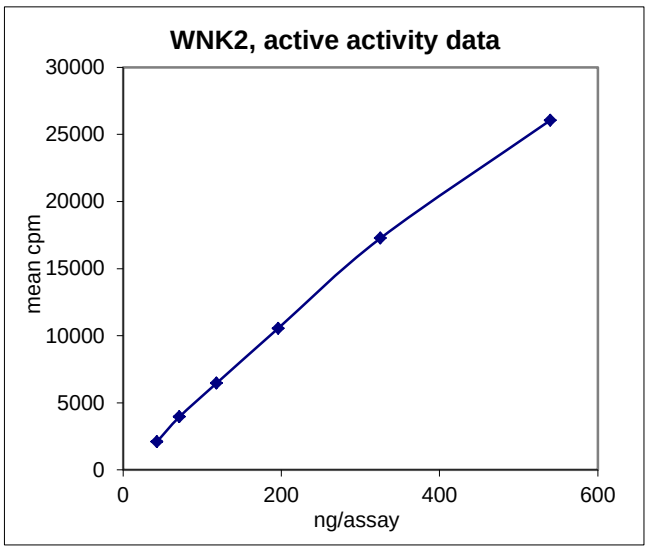
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

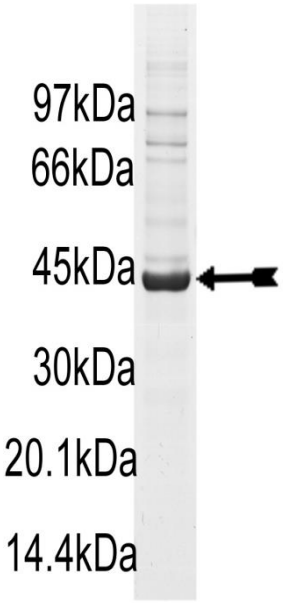
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 43–540ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein 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 WNK2 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of WNK2, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom

T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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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 assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
3. **WNK2, active:** Dilute with 20mM MOPS-NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 43–540ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 (43–540ng) WNK2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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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WNK2 Sequence Information

Protein human WNK2

Tags *N*-term 6His

Native sequence G31 of recombinant sequence is equivalent to G166 of native human WNK2

Accession number GenBank NM_006648

Recombinant WNK2 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF GRTRRDEPEE EEDDEDDLKA VATSLDGRFL
  61 KFDIELGRGS FKTVYKGLDT ETWVEVAWCE LQDRKLTKLE RQRFKEEAEM LKGLQHPNIV
 121 RFYDFWESSA KGKRCIVLVT ELMTSGTLKT YLKRFKVMKP KVLRSWCRQI LKGLLFLHTR
 181 TPPIIHRDLK CDNIFITGPT GSVKIGDLGL ATLKRASFAK SVIGTPEFMA PEMYEEHYDE
 241 SVDVYAFGMC MLEMATSEYP YSECQNAAQI YRKVTCGIKP ASFEKVHDPE IKEIIGECIC
 301 KNKEERYEIK DLLSHAFFAE DTGVRVELAE EDHGRKSTIA LRLWVEDPKK LKGK
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Recombinant WNK2 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc gggcgcactc gccgggacga gcccgaagag
 121 gaggaggacg acgaggacga cctcaaggcc gtggccacct ctctggacgg ccgcttcctc
 181 aagttcgaca tcgagctggg ccgcggttcc ttcaagacgg tctacaaggg gctggacacg
 241 gagacctggg tggaggtggc ctggtgtgag ctgcaggacc ggaagctcac caagctggag
 301 cggcagcggt tcaaggaaga ggctgagatg ctgaaaggcc tgcagcaccc caacatcgtg
 361 cgcttctacg acttctggga gtccagcgcc aagggcaagc ggtgcattgt gctggtgacg
 421 gagctgatga cctcagggac gctgaagaca tacctgaagc ggttcaaggt gatgaagccc
 481 aaggttctcc gcagctggtg ccggcagatc ctgaagggcc tgctgttcct gcacacaagg
 541 acgccaccca tcatccaccg agacctgaaa tgtgacaata ttttcatcac cggaccaact
 601 gggtctgtga agattggcga cttgggcctg gccactctga aaagagcgtc atttgccaaa
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1021 ctgaggctct gggtggaaga ccccaagaaa ctgaagggaa agtaa
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Reviewed and approved by site quality representative.