

Certificate of Analysis

VRK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-732, 14-732-K, 14-732M

Parent Lot # WAE0478

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 VRK2 amino acids 1–336, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 43.0kDa.

Specific Activity (Parent lot# WAE0478): 52U/mg, where one unit of VRK2, active activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.60mg/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.

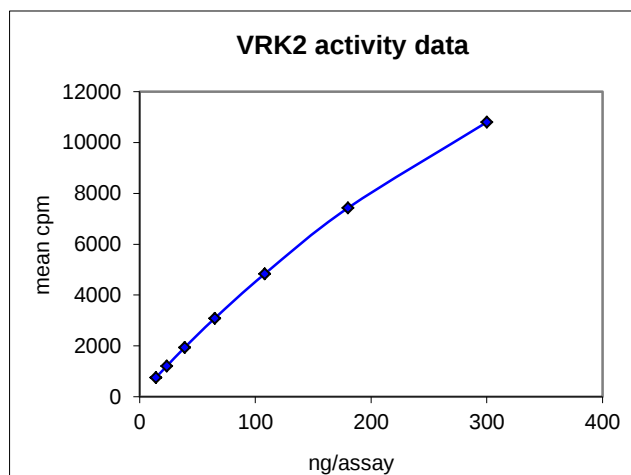
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

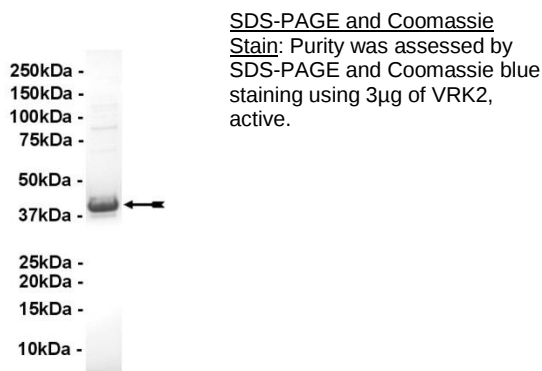
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 14–300ng of this lot of enzyme phosphorylated 2mg/ml casein 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 product identity as VRK2 with the translated 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. **Casein:** Use at a final assay concentration of 2mg/ml. Prepare a 20mg/ml stock and add 2.5µl of stock per assay point.
3. **VRK2, 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 14–300ng 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 assay):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **Casein**.
3. Add **2.5µl (14–300ng) VRK2, 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 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 enzyme components plus 1µl 30% phosphoric acid.

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VRK2 Sequence Information

<u>Protein</u>	human VRK2
<u>Tags</u>	N-term 6His
<u>Native sequence</u>	M40 of recombinant sequence is equivalent to M1 of native human VRK2
<u>Accession number</u>	GenBank NM_006296

Recombinant VRK2 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQPEVM PPKRNEKYKL PIPFPEGKVL
61 DDMEGNQWVL GKKIGSGGFG LIYLAFPTNK PEKDARHVVK VEYQENGPLF SELKFYQ RVA
121 KKDCIKKWIE RKQLDYL GIP LFYGSGLTEF KGRSYRFMVM ERLGIDLQKI SGQNGTFKKS
181 TVLQLGIRML DVLEYIHENE YVHGDVKAAN LLLGYKNPDQ VYLADYGLSY RYCPNGNHKQ
241 YQENPRKGHN GTIEFTSLDA HKGVALSRRS DVEILGYCML RWLCGKLPWE QNLKDPVAVQ
301 TAKTNLLDEL PQSVLKWAPS GSSCCEIAQF LVCAHSLAYD EKPNYQALKK ILNPHGIPLG
361 PLDFSTKGQS INVHT

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Recombinant VRK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc aaaggcctac gtcgacagcc cgaagtgatg
121 ccaccaaaaa gaaatgaaaa atacaaactt cctattccat ttccagaagg caaggttctg
181 gatgatattg aaggcaatca gtgggtactg ggcaagaaga ttggctctgg aggatttgga
241 ttgatataatt tagctttccc cacaaataaa ccagagaaaag atgcaagaca ttagtagtaaaa
301 gtggaatatc aagaaaatgg cccgttattt tcagaactta aattttatca gagagttgca
361 aaaaaagact gtatcaaaaa gtggatagaa cgcaaacac ttgattattt aggaattcct
421 ctgttttatg gatctggtct gactgaattc aagggaagaa gttacagatt tatggtaatg
481 gaaagactag gaatagattt acagaagatc tcaggccaga atggtacctt taaaaagtca
541 actgtcctgc aattaggatc ccgaatgttg gatgtactgg aatatataca tgaaaatgaa
601 tatgttcatt gtgatgtaaa agcagcaaat ctacttttgg gttacaaaaa tccagaccag
661 gtttatcttg cagattatgg actttcctac agatattgtc ccaatgggaa ccacaaacag
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1081 ccactggact tttccacaaa aggacagagt ataaatgtcc atacttaa

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