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Certificate of Analysis

Raf-1 (truncated), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-352, 14-352-K, 14-352M
Parent Lot # WAA0188

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 GST-tagged recombinant human Raf-1 residues 306–end, containing the mutations Y340D and Y341D. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 89.8% by SDS-PAGE and Coomassie blue staining. MW = 65kDa.

Specific Activity (Parent lot# WAA0188): 113212U/mg, where one unit of Raf-1 activity = 1 unit of MAPK2 (cat# 14-198) activity which in turn is defined as 1nmol phosphate incorporated into 0.33mg/ml MBP per minute at 30°C with a final ATP concentration of 100µM. Note the activity is determined using a triple linked assay which involves the activation of MEK1 (cat# 14-420) by RAF-1, followed by the subsequent activation of MAPK2 (cat# 14-198) by the activated MEK1.

Formulation: 0.564mg/ml of enzyme in 50mM Tris/HCl, pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

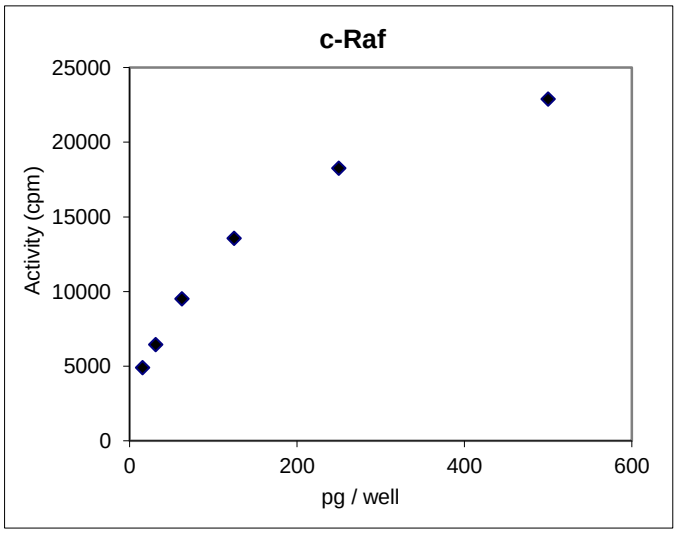
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

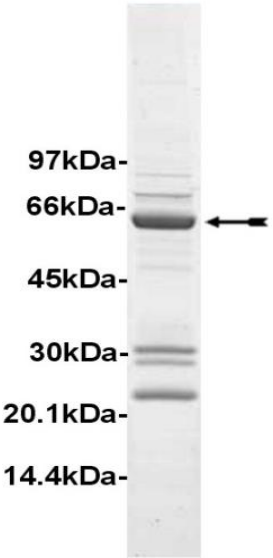
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 16–500pg of this lot of enzyme was used to activate 0.2µM MEK1 (cat# 14-420) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results.



MS Tryptic Fingerprint: Confirmed product identity as Raf-1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of RAF-1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 1% 2-mercaptoethanol, 0.1% Brij-35.
2. **MEK1 (Catalogue # 14-420), unactive:** Use at a final assay concentration of 0.2µM (0.0126mg/ml). Prepare a 0.126mg/ml stock and add 2.5µl of stock per assay point.
3. **MAPK2 (Catalogue # 14-198), unactive:** Use at a final assay concentration of 2µM (0.136mg/ml). Prepare a 1.36mg/ml stock and add 2.5µl of stock per assay point.
4. **Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
5. **Raf-1, active:** Dilute with 25mM Tris/HCl pH7.5, 0.1mM EGTA, 1mg/ml BSA. Use 16–500pg per assay point.
6. **[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 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **MEK1, unactive**.
3. Add 2.5µl of **MAPK2, unactive**.
4. Add 2.5µl of **MBP**.
5. Add **5µl (16–500pg) Raf-1, active**.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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Raf-1 Sequence Information

Protein Human Raf-1 Y340D Y341D, truncated

Accession number EMBL X03484

Tags *N*-terminal GST

Native sequence S226 of the recombinant protein is equivalent to S306 of native sequence. Y340 and Y341 of the native Raf-1 sequence have been mutated to aspartate to provide constitutive activation of the enzyme.

Recombinant RAF-1 amino acid sequence:

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSQPKT PVPAQRERAP
241 VSGTQEKNKI RPRGQRDSSD DWEIEASEVM LSTRIGSGSF GTVYKGKWHG DVAVKILKVV
301 DPTPEQFQAF RNEVAVLRKT RHVNILLFMG YMTKDNLAIV TQWCEGSSLY KHLHVQETKF
361 QMFQLIDIAR QTAQGMDYLH AKNIIHRDMK SNNIFLHEGL TVKIGDFGLA TVKSRWSGSQ
421 QVEQPTGSVL WMAPEVIRMQ DNNPFSFQSD VYSYGIVLYE LMTGELPYSH INNRDQIIFM
481 VGRGYASPDL SKLYKNCPKA MKRLVADCVK KVKEERPLFP QILSSIELLQ HSLPKINRSA
541 SEPSLHRAAH TEDINACTLT TSPRLPVF
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Recombinant RAF-1 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttca aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatccca gccgaaaacc cccgtgccag cacaaagaga gcgggcacca
 721 gtatctggga cccaggagaa aaacaaaatt aggcctcgtg gacagagaga ttcaagcgat
 781 gattgggaaa tagaagccag tgaagtgatg ctgtccactc ggattgggtc aggctctttt
 841 ggaactgttt ataagggtaa atggcacgga gatgttgcag taaagatcct aaaggttgtc
 901 gacccaaccc cagagcaatt ccaggccttc aggaatgagg tggctgttct gcgcaaaaca
 961 cggcatgtga acattctgct tttcatgggg tacatgacaa aggacaacct ggcaattgtg
1021 acccagtggt gcgagggcag cagcctctac aaacacctgc atgtccagga gaccaagttt
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1141 gcaaagaaca tcatccatag agacatgaaa tccaacaata tatttctcca tgaaggctta
1201 acagtgaaaa ttggagattt tggtttggca acagtaaagt cacgctggag tggttctcag
1261 caggttgaac aacctactgg ctctgtcctc tggatggccc cagaggtgat ccgaatgcag
1321 gataacaacc cattcagttt ccagtcggat gtctactcct atggcatcgt attgtatgaa
1381 ctgatgacgg gggagcttcc ttattctcac atcaacaacc gagatcagat catcttcatg
1441 gtgggccgag gatatgcctc cccagatctt agtaagctat ataagaactg ccccaaagca
1501 atgaagaggc tggtagctga ctgtgtgaag aaagtaaagg aagagaggcc tctttttccc
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1561 cagatcctgt cttccattga gctgctccaa cactctctac cgaagatcaa ccggagcgct
1621 tccgagccat ccttgcatcg ggcagcccac actgaggata tcaatgcttg cacgctgacc
1681 acgtccccga ggctgcctgt cttctag
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Reviewed and approved by site quality representative.