

## 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.

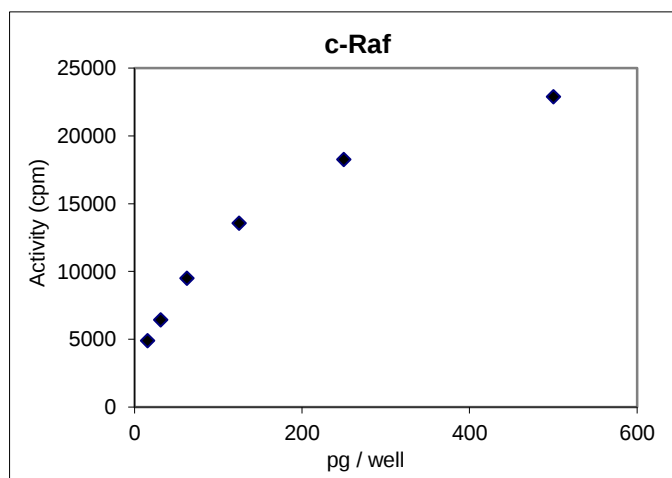
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

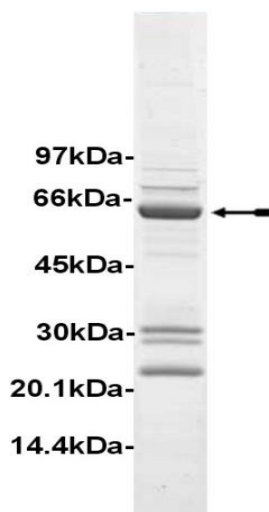
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 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<sub>3</sub>VO<sub>4</sub>, 1% 2-mercaptoethanol, 0.1% Brij-35.
2. **MEK1 (Catalogue # 14-420), inactive:** 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), inactive:** 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-<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[γ-<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-<sup>33</sup>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, inactive**.
3. Add 2.5µl of **MAPK2, inactive**.
4. Add 2.5µl of **MBP**.
5. Add **5µl (16–500pg) Raf-1, active**.
6. Add 10µl of diluted [γ-<sup>33</sup>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

|                                |                                                                                                                                                                                                       |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Protein</u></b>          | Human Raf-1 Y340D Y341D, truncated                                                                                                                                                                    |
| <b><u>Accession number</u></b> | EMBL X03484                                                                                                                                                                                           |
| <b><u>Tags</u></b>             | N-terminal GST                                                                                                                                                                                        |
| <b><u>Native sequence</u></b>  | 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:

```

1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSM A IIRYIADKH N MLGGCPKERA EISMLEGAVL DIRYGVSR I A YSKDFETLKV
121 DFLSKLPEM L KMFKDRLCH K TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSQPKT PVPAQRERAP
241 VSGTQEKNKI RPRGQRDSSD DWEIEASEVM LSTRIGSGSF GTVYKGKWHG DVAVKILKVV
301 DPTPEQFQAF RNEVAVLRKT RHVNILLFMG YMTKDNLAIV TQWCEGSSLY KHLHVQETKF
361 QMFQLIDIAR QTAQGM DY LH AKNIIHRDMK SNNIFLHEGL TVKIGDFGLA TVKSRWSGSQ
421 QVEQPTGSVL WMAPEVIRMQ DNNPFSFQSD VYSYGIVLYE LMTGELPYSH INN RDQIIFM
481 VGRGYASPD L SKLYKNCPKA MKRLVADCVK KVKEERPLFP QILSSI ELLQ HSLPKINRSA
541 SEPSLHRAAH TEDINACTLT TSPRLPVF

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## Recombinant RAF-1 nucleotide sequence:

```

1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttca aagatcgttt atgtcataaa
421 acatatatta atggtgatca tgtaacccat cctgacttca tgttgatatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc aaaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatccca gccgaaaacc cccgtgccag cacaagaga gcgggcacca
721 gtatctggga cccaggagaa aaacaaaatt aggcctcgtg gacagagaga ttcaagcgat
781 gattgggaaa tagaagccag tgaagtgat ctgtccactc ggattgggtc aggcctctttt
841 ggaactgttt ataagggtaa atggcacgga gatgttgtag taaagatcct aaaggttgct
901 gacccaacc cagagcaatt ccaggccttc aggaatgagg tggctgttct gcgcaaaaca
961 cggcatgtga acattctgct tttcatgggg tacatgacaa aggacaacct ggcaattgtg
1021 acccagtggt gcgagggcag cagcctctac aaacacctgc atgtccagga gaccaagttt
1081 cagatgttcc agctaattga cattgcccg gacacggctc agggaaatgga ctatttgcat
1141 gcaagaaca tcatccatag agacatgaaa tccaacaata ttttctcca tgaaggctta
1201 acagtgaaaa ttggagattt tggtttgcca 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 cttgcatcg ggcagcccac actgaggata tcaatgcttg cacgctgacc
1681 acgtccccga ggctgcctgt cttctag
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