

Certificate of Analysis

B-Raf, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-530, 14-530-K, 14-530M

Parent Lot # 2472881

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 B-Raf residues 416–end. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 46.3% by SDS-PAGE and Coomassie blue staining. MW = 67.2kDa.

Specific Activity (Parent lot# 2472881): 142629U/mg, where one unit of B-Raf = 1unit of MAPK2(m) activity which in turn is equivalent to 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM. Note the activity is determined by a triple linked assay which involves the activation of MEK1(h) (cat# 14-420) by B-Raf, followed by the subsequent activation of MAPK2(m) (cat# 14-198) by the activated MEK1(h).

Formulation: 0.527mg/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.

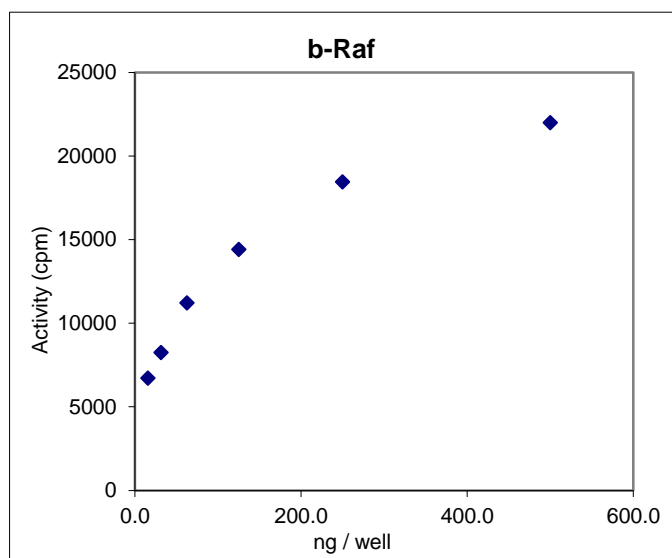
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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(h) 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 B-Raf with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solution

- 1. 10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mMNa₃VO₄, 1% 2-mercaptoethanol, 0.1% Brij-35.
- 2. MEK1(h), unactive (cat# 14-420):** Use at a final assay concentration of 0.2µM (0.0126mg/ml). Prepare a 0.126g/ml stock and add 2.5µl of stock per assay point.
- 3. MAPK2(m), unactive (cat# 14-198):** Use at a final assay concentration of 2µM (0.14 mg/ml). Prepare a 0.7mg/ml stock and add 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. B-Raf, active:** Dilute with 25mM Tris/HCl pH7.5, 0.1mM EGTA, 1mg/ml BSA. Use 16–500pg per assay point.
- 6. [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 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **MEK1(h), unactive**.
3. Add 5µl of **MAPK2(m), unactive**.
4. Add 2.5µl of **MBP**.
5. Add **5µl (6–500pg) B-Raf, active**.
6. Add 10 µl of diluted [γ-³³P] 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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B-Raf Sequence Information

<u>Protein</u>	Human B-Raf
<u>Tags</u>	N-Terminal GST
<u>Native sequence</u>	Q237 of the fusion protein is equivalent to Q416 of human B-RAF
<u>Accession number</u>	GenBank NM_004333

Recombinant B-RAF amino acid sequence:

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1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLP EML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKS SKYIA WPLQGWQATF GGGDHPPKSD LEVL FQGPEF KGLRRQ QKSP
241 GPQREKSSS SSEDNRMKT LGRRDSSDDW EIPDQGITVG QRIGSGSFGT VYK GKWHGDV
301 AVKMLNVTAP TPQQLQAFKN EVGVL RKRTRH VNILLFMGYS TKPQLAIVTQ WCEGSSLYHH
361 LHI IETKFEM IKLID IARQT AQGMDYLHAK SIIHRDLKSN NIFLHEDLTV KIGDFGLATV
421 KSRWSGSHQF EQLSGSILWM APEVIRMQDK NPYSFQSDVY AFGIVLYELM TGQLPYSNIN
481 NRDQIIFMVG RGYLSPDLSK VRSNCPKAMK RLMAECLKKK RDERPLFPQI LASIELLARS
541 LPKIHR SASE PSLNRAGFQT EDFSLYACAS PKTPIQAGGY GAFPVH
  
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Recombinant B-RAF nucleotide sequence:

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1 atgtccccta tactaggtta ttgga aaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag cttttgtatg agcgcgatga aggtgataaa
121 tggcga aaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatg tta aataacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgaacccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggaagttc tgttccaggg gcccgattc aaaggcctac gtcgacaaca gaaatctcca
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1261 aaatctcgat ggagtgggtc ccatcagttt gaacagttgt ctggatccat tttgtggatg
1321 gcaccagaag tcatcagaat gcaagataaa aatccataca gctttcagtc agatgtatat
1381 gcatttggaa ttgttctgta tgaattgatg actggacagt taccttattc aaacatcaac
1441 aacagggacc agataat ttt tatggtggga cgaggatacc tgtctccaga tctcagtaag
1501 gtacggagta actgtccaaa agccatgaag agattaatgg cagagtgcct caaaaagaaa
1561 agagatgaga gaccactctt tcccaaatt ctcgcctcta ttgagctgct ggcccgtcta
  
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1621 ttgcaaaaa ttcaccgag tgcacagaa ccctccttga atcgggctgg tttcaaaaca  
1681 gaggatttta gtctatatgc ttgtgcttct ccaaaaacac ccatccaggc aggggatat  
1741 ggtgcgtttc ctgtccactg a
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