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Product Information

BRAF (416-766), active, GST tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **B4062**
Storage Temperature –70 °C

Synonyms: BRAF1, RAFB1, B-raf 1, MGC126806, MGC138284

Product Description

BRAF is a member of the RAF family that is activated by members of the Ras family upon growth factor-induced stimulation. Active Ras can induce heterodimerization of cRaf and BRAF, and this may explain the observed cooperativity of cRaf and BRAF in cells responding to growth factor signals.[1] Activating mutations in the *BRAF* gene are present in a large percentage of human malignant melanomas and in a proportion of colon cancers. The vast majority of these mutations result in a valine to glutamic acid change at residue 599 within the activation segment of BRAF.[2]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST-tag. The gene accession number is NM 004333. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~63 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot:
≥70% (SDS-PAGE, densitometry)

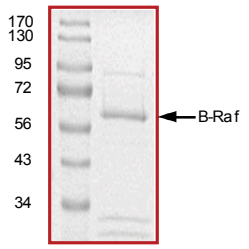
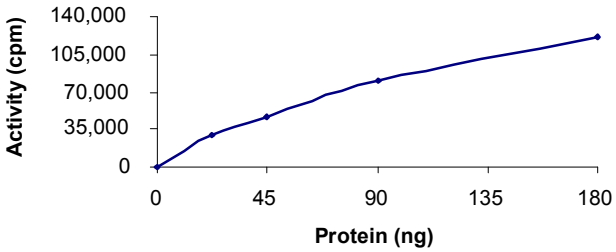


Figure 2.
Specific Activity of Typical Lot:
63–85 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µL BSA and 5% glycerol solution.

Kinase Solution – Dilute the active BRAF (0.1 μg/μL) with Kinase Dilution Buffer to the desired concentration. Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active BRAF kinase for optimal results

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μL aliquots at –20 °C.

γ-^{32}P-ATP Assay Cocktail (250 μM) – Combine 5.75 ml of Kinase Assay Buffer, 150 μL of 10 mM ATP Stock Solution, 100 μL of γ-^{32}P-ATP (1 mCi/100 μL). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the inactive MEK1 in water at a final concentration of 0.2 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active BRAF, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{32}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μL:
   10 μL of Kinase Solution
   10 μL of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 μL of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μL of the γ-^{32}P-ATP Assay Cocktail, bringing the final reaction volume to 25 μL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μL of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{32}P-ATP counts introduced into the reaction. Spot 5 μL of the γ-^{32}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μL of γ-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μL of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Weber, C.K. et al., Active Ras induces heterodimerization of cRaf and BRaf. Cancer Res., **61**(9), 3595-8 (2001).
2. Mercer, K.E. et al., Raf proteins and cancer: B-Raf is identified as a mutational target. Biochim. Biophys. Acta, **1653**(1), 25-40 (2003).

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