



Catalog # Aliquot Size

B08-12GG -05 5 µg

B08-12GG -10 10 µg

## BRAF (G466V) Protein

Recombinant human protein expressed in Sf9 cells

Catalog # B08-12GG

Lot # K1803-3

### Product Description

Recombinant human BRAF (G466V) (381-end or exon 9-18) protein was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_004333](#).

### Gene Aliases

BRAF (Ex9-18), BRAF1, RAFB1, B-raf, B-raf 1, MGC126806, MGC138284

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

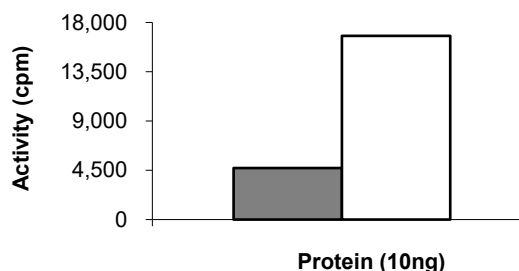
### Scientific Background

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).

### References

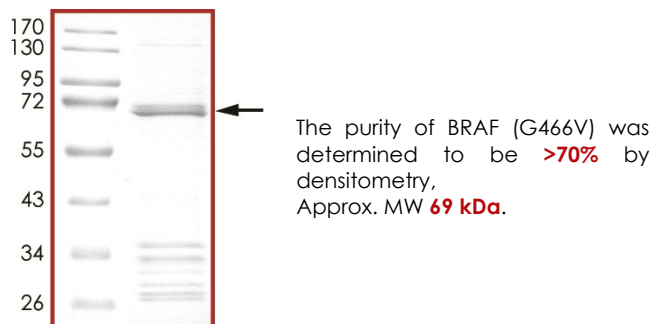
1. Weber, C K. et al: Active Ras induces heterodimerization of cRaf and Braf. *Cancer Res.* 2001 May 1;61(9):3595-8.
2. Mercer, K E. et al: Raf proteins and cancer: B-Raf is identified as a mutational target. *Biochim Biophys Acta.* 2003 Jun 5;1653(1):25-40.

### Specific Activity



BRAF (G466V) activity assay with or without downstream substrate inactive MEK1-ERK1. This recombinant BRAF mutant did not show significant kinase activity *in vitro* using the activity assay protocol in the data sheet.

### Purity



## BRAF (G466V) Protein

Recombinant human protein expressed in Sf9 cells

Catalog #	B08-12GG
Lot #	K1803-3
Purity	>70%
Concentration	0.2 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: B08-12GG)

BRAF (G466V) (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of BRAF for optimal results).

### Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA.

### Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: M02-14BG)

Unactive MEK1 (Catalog #: M02-14BG) and ERK1 (Catalog #: M29-14G) were activated in a coupled reaction. Myelin Basic Protein (MBP) (Catalog #: M42-51N) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml was subsequently used as a substrate for the activated ERK1.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the BRAF (G466V), Kinase Assay Buffer, Unactive ERK1 and Unactive MEK1 on ice. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1.** 10µl of diluted BRAF (G466V) (Catalog #B08-12GG)
  - Component 2.** 0.25µl of Unactive MEK1 (0.2µg/µl) (Catalog #M02-14BG)
  - Component 3.** 0.25µl of Unactive ERK1 (0.2µg/µl) (Catalog #M29-14G)
  - Component 4.** 4.5µl of Kinase Dilution Buffer (Catalog #K23-09)
- Step 3.** Start the reaction by the addition of 5 µl [<sup>33</sup>P]-ATP Assay Cocktail solution and incubate in a water bath at 30°C for 15 minutes.
- Step 4.** After the 15 minute incubation period, add 5µl of MBP substrate on ice (1 mg/ml) (Catalog #M42-51N) bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 5.** Set up the blank control as outlined in step 4, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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# SAFETY DATA SHEET

## Article 1 – Product Identification

**Product Name: BRAF (G466V) Protein**

**Catalog # B08-12GG**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name: SignalChem Biotech Inc.  
 Street Address: 110-13120 Vanier Place  
 City, Prov. Postal Code: Richmond, BC, V6V 2J2  
 Fax: 604-232-4601  
 EMERGENCY PHONE: 604-232-4600

## Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

## Article 3 – Composition/Information on Ingredients

**Chemical Characterization:** Mixtures.

**Description:** This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	≤25%
NaCl	Sodium chloride	7647-14-5	≤1.753 %
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 - Amino - 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	1185-53-1	<0.8%
Glutathione	Glutathione	70-18-8	0.307%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
EDTA	Ethylenediaminetetraacetic acid	6381-92-6	0.0037%
PMSF; Phenylmethanesulfonyl fluoride	α-Toluenesulphonyl fluoride	329-98-6	0.002%

## Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

## Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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## Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

## Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

## Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**  
Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m <sup>3</sup> for mist	British Columbia, Canada
TWA	3 mg/m <sup>3</sup> for respirable mist	British Columbia, Canada
TWA	10 mg/m <sup>3</sup>	Alberta, Canada
TWAEV	10 mg/m <sup>3</sup>	Ontario, Canada
TWAEV	10 mg/m <sup>3</sup>	Quebec, Canada
TWA	10 mg/m <sup>3</sup>	USA

- **Appropriate engineering controls:**  
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**  
**Respiratory protection:**  
Use appropriate respirator if there is inadequate ventilation by following the government standards.  
**Hand protection:**  
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.  
**Eye/face protection:**  
Safety goggles with side-shields approved under appropriate government standards.  
**Skin/body protection:**  
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

## Article 9 – Physical and Chemical Properties

<b>Appearance:</b> Colorless fluid.	<b>Danger of explosion:</b> Product does not present an explosion hazard.
<b>Odour/Odour Threshold:</b> Not determined.	<b>Explosion limits:</b> Lower: 0.9 Vol %; Upper: 0.0 Vol %.
<b>pH:</b> Not available.	<b>Decomposition temperature:</b> Not available.
<b>Melting point/freezing point:</b> Not determined.	<b>Vapor pressure at 20 °C:</b> 0.1 hPa
<b>Boiling point/Boiling range:</b> 100 °C.	<b>Density:</b> Not determined.
<b>Flash point:</b> > 100 °C.	<b>Relative density:</b> Not determined.
<b>Flammability (solid, gaseous):</b> Not determined.	<b>Vapor density:</b> Not determined.
<b>Ignition temperature:</b> 400 °C.	<b>Evaporation rate:</b> Not determined.
<b>Auto-igniting:</b> Product is not self-igniting.	<b>Solubility in / Miscibility with Water:</b> Fully miscible.

## Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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## Article 11 - Toxicological Information

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- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**  
**Inhalation:** May be harmful if inhaled. May cause respiratory tract irritation.  
**Ingestion:** May be harmful if swallowed.  
**Skin:** May be harmful if absorbed through skin. May cause skin irritation.  
**Eyes:** May cause eye irritation.
- **Signs and Symptoms of Exposure:**  
Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

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## Article 12 - Ecological Information

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- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

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## Article 13 - Disposal Considerations

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- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

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## Article 14 - Transport Information

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- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

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## Article 15 - Regulatory Information

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- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

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## Article 16 - Other Information

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The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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