

## Human BRAF ELISA Kit

### Catalog No. EA200054

#### Principle of the Assay

B-Raf protein is encoded by BRAF gene in human. The gene is also referred to as proto-oncogene B-Raf. B-Raf is a serine/threonine-specific protein kinase. In its active conformation, B-Raf forms dimers via hydrogen-bonding and electrostatic interactions of its kinase domains. The B-Raf protein is involved in sending signals inside cells which are involved in directing cell growth. More than 30 mutations of the BRAF gene associated with human cancers have been identified. Mutations in this gene have been found in cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, papillary thyroid carcinoma, non-small-cell lung carcinoma, adenocarcinoma of the lung, brain tumors including glioblastoma and pleomorphic xanthoastrocytoma as well as inflammatory diseases like Erdheim-Chester disease. The V600E mutation of the BRAF gene has been associated with hairy cell leukemia.

This sandwich ELISA is used to measure human BRAF in serum, plasma, and other biological fluids. Microtitration wells coated with anti-human BRAF capture antibody are exposed to test specimens. The BRAF antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured BRAF antigen is then reacted with a biotinylated human BRAF detection antibody. Subsequently, Streptavidin-HRP conjugate is then added. After wash, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of BRAF present in a sample.

#### Materials Supplied

Description	Quantity
BRAF Monoclonal Antibody Coated 96-well Plate in foil pouch with desiccant	1
Recombinant Human BRAF Protein (1000ng/mL)	0.1 mL
Biotinylated BRAF Monoclonal Antibody (100x)	120 µL
Streptavidin -HRP Conjugate (100x)	120 µL
Sample Diluent	30 mL
Assay Buffer	40 mL
Substrate Solution (TMB)	12 mL
Stop Solution (1N HCl)	12 mL
Wash Buffer (20x)	60 mL
Plate Sealer	3

#### Additional Materials not Supplied

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.

2. Disposable tip micropipettes to deliver volumes of 5µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 10mL.
5. Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder, 1 mL, 5 mL, and 10 mL pipettes.
6. Absorbent paper towels.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.
9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

#### Storage and Stability

Upon receipt, store the kit at 2-8°C. The kit should not be used beyond the expiration date. Once opened, the unused microplate strips should be returned to their original foil pouch along with the desiccant. The diluted Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the diluted Wash buffer becomes visibly cloudy during the 3 weeks, discard it. (Note: Concentrated Wash Buffer, when stored at 2-8°C, normally may develop crystalline precipitates, which can be re-dissolved at 37°C.)

#### *Indications of Deterioration*

The human BRAF Assay kit may be considered to have deteriorated if:

1. Reagents are visibly cloudy.
2. The Substrate Solution turns blue. This is likely to be caused by chemical contamination of the Substrate Solution.

#### Precautions

1. The reagents supplied in this kit are for **Research use only**.
2. All blood products should be treated as potentially infectious.
3. Disposal or decontamination of fluid in the waste reservoir should be in accordance with guidelines described in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910.1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.
4. The Substrate Solution and Stop Solution in this kit can irritate the skin and cause eye damage. Handle them with care and wear protective gloves, clothing and eye/face protection. Wash hands thoroughly after handling. Immediately flush the affected area with plenty of water in case of contact with skin or eyes. Obtain medical attention if necessary.

#### Technical Suggestions

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use the kit after the expiration date printed on the outer carton label.

3. Do not cross contaminate reagents.
4. Some reagents in the BRAF ELISA kit are optimized for each kit lot. Do not exchange reagents from kits with different lot numbers.
5. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays. Always use fresh pipette tips when drawing from stock reagent bottles.
6. Warm up the foil bag to room temperature before opening.
7. All reagents should be added to the plate in the same order.
8. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).
9. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. For automatic processors, follow manufacturer's recommendations.
10. It is recommended that all pipetting devices (manual or automatic), and thermometers are regularly calibrated according to the manufacturer's instructions.

### **Sample Collection and Storage**

The Human BRAF ELISA is intended for use with serum, plasma, and other biological fluids. The specimen should be tested as soon as possible. However, if the specimen needs storage, the specimens should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Specimens that have been frozen and thawed should be thoroughly mixed before testing.

**Cell Culture Supernatants** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq$  -20 °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq$  -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq$  -20 °C. Avoid repeated freeze-thaw cycles.

**Cell Lysates** - Collect and pellet the cells by centrifugation and remove the supernatant. Wash the cells 2 times with PBS. Solubilize cells at  $1 \times 10^7$  cells/mL in cell lysis buffer (25mM Tris-HCl pH7.6, 150mM NaCl, 1% NP-40, 1mM EDTA, 1xProteinase inhibitor cocktail mix (Sigma), 1mM PMSF and 1mM Na3VO4) and allow samples to sit on ice for 15 minutes. Assay immediately or store at  $\leq$  -70 °C. Before use, centrifuge samples at 2000 x g for 5 minutes, and transfer the supernatant to a clean test tube. Sample protein concentration may be quantified using a total protein assay. If needed, further dilutions should be made in Sample Diluent.

### **Rinse Cycle**

Aspirate each well and wash. Wash by filling each well with Wash Buffer (300 $\mu$ L) using a squirt bottle, manifold dispenser, or automatic plate washer. Complete removal of liquid at each

step is essential to good performance. After the last wash, invert the plate and blot it against clean paper towels.

### **Preparation for the Assay**

**1. Standard preparation:** Prepare protein standard by diluting 10 $\mu$ L of standard stock into 490  $\mu$ L (1:50 dilution) of **Assay Buffer**. This will give a final concentration of 20ng/mL as shown in Table 1. Make 2x serial dilution of Standard 1 using **Assay Buffer**, to generate a standard concentration range of 0.31 to 20ng/mL.

**2. Sample preparation:** BRAF concentration must be estimated prior to performing the full experiment by testing a serially diluted representative sample using **Sample Diluent**. Select an optimal dilution level such that the final target protein concentration falls near the middle of the assay linear dynamic range.

**3. Detection antibody preparation:** dilute the concentrated biotin conjugated detection antibody 1:100 using **Assay Buffer**.

**4. SA-HRP preparation:** dilute the concentrated streptavidin HRP conjugate 1:100 using **Assay Buffer**.

**5. Wash buffer:** Prepare working-strength Wash buffer by diluting 1 part concentrate with 19 parts of distilled or deionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs.

**Table 1: Human BRAF Standard Curve Generation**

Standard Number	Concentration of BRAF (ng/mL)	BRAF Standard ( $\mu$ L)	Assay Buffer ( $\mu$ L)
1	20	10	490
2	10	250 of #1	250
3	5	250 of #2	250
4	2.5	250 of #3	250
5	1.25	250 of #4	250
6	0.625	250 of #5	250
7	0.3125	250 of #6	250
8	0		250

### **Assay Procedure**

**Note: All standards, controls and samples should be tested in duplicate.**

1. Allow all reagents to reach room temperature (18-25°C).
2. Select sufficient microtitration well strips to accommodate all test specimens, controls and reagent blank. Fit the strips into the holding frame.
3. Dispense 100  $\mu$ L of each standard and sample into appropriate wells.
4. Incubate for 2 hours at room temperature with moderate shaking (450 $\pm$ 50rpm) on a horizontal orbital plate shaker.

5. Wash the microtitration plate 3 times as described in the Rinse Cycle section.
6. Add 100  $\mu$ L of working concentration detection antibody into each well and incubate for 1.5 hour at room temperature with moderate shaking (450 $\pm$ 50rpm) on a horizontal orbital plate shaker.
7. Wash the microtitration plate 3 times as described in the Rinse Cycle section.
8. Add 100  $\mu$ L of working concentration Streptavidin HRP conjugate into each well and incubate for 25 minutes at room temperature with moderate shaking (450 $\pm$ 50rpm) on a horizontal orbital plate shaker.
11. Wash the microtitration plate 5 times as described in the Rinse Cycle section.
12. Dispense 100  $\mu$ L Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) and protected from direct sunlight for 20-25 minutes.
13. Stop the reaction by adding 100  $\mu$ L of Stop Solution to each well. The blue solution should change to a uniform yellow color. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.
14. Read the absorbance values at 450 nm using a microplate reader. If wavelength correction is available, set to 540 nm or 570 nm.

### Calculation of Results

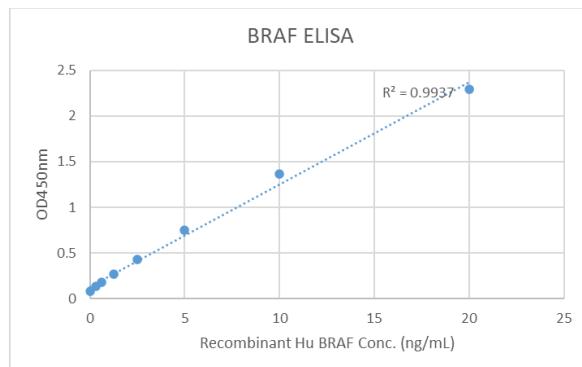
Average the duplicate readings for each standard and sample. A 4-parameter logistic (4-PL) or a linear regression model providing a point-to-point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or a linear regression curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Do not force the line to be linear. The concentration of the samples can be found directly from the standard curve.

**Table 2. Example Data at 450nm.**

Standards	450 nm absorbance
Standard 1 (20ng/mL)	2.29305
Standard 2 (10ng/mL)	1.36465
Standard 3 (5ng/mL)	0.74625
Standard 4 (2.5ng/mL)	0.43265
Standard 5 (1.25ng/mL)	0.2721
Standard 6 (0.625ng/mL)	0.1792
Standard 7 (0.3125ng/mL)	0.13015
Standard 8 (0 ng/mL)	0.08055

### Typical Human BRAF ELISA Kit Standard Curve

This standard curve was generated at OriGene for demonstration purpose only.



Note: This standard curve is only an example and should not be used to generate any results.

**Assay Sensitivity:** 0.115ng/mL

### Limitations of Use

1. This kit is for research use only, **not for use in diagnostic procedures.**
2. The BRAF value measured using OriGene BRAF ELISA kit may not be interchangeable with that obtained from other assay kits.
3. The assay cannot be used to quantitate samples with values higher than the highest standard without further dilution of the samples.

**Assay Flowchart**

**Add 100  $\mu$ L of Stds and Samples, incubate 2 hrs at RT with Shaking**

↓ Wash 3 times

**Add 100  $\mu$ L of Detection Antibody  
Incubate 1.5 hour at RT with Shaking**

↓ Wash 3 times

**Add 100  $\mu$ L of SA-HRP  
Incubate 25 minutes at RT with Shaking**

↓ Wash 5 times

**Add 100  $\mu$ L of TMB Substrate  
Incubate 20-25 minutes at RT**

↓

**Add 100  $\mu$ L of Stop Solution**

↓

**Read OD450nm in Plate Reader**