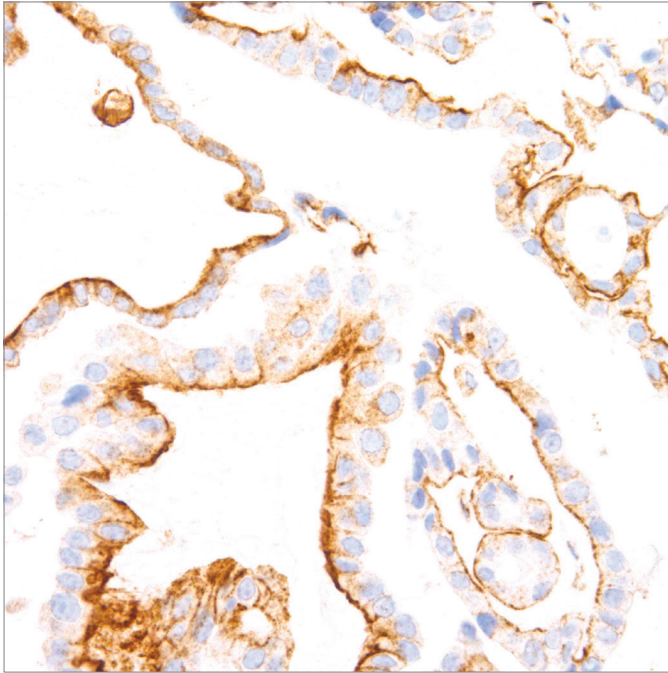


GeneAb™

BRAF V600E

Research Use Only

Clone: IHC600 | Source: Mouse Monoclonal | Positive Control: Colorectal Adenocarcinoma



GeneAb™ BRAF V600E (IHC600) on Thyroid Gland

1. Intended Use

The BRAF V600E (IHC600) antibody is intended for qualified laboratories to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-fixed, paraffin-embedded tissue sections using IHC test methods.

2. Summary and Explanation

Serine/threonine-protein kinase B-Raf (BRAF) is a cytoplasmic serine-threonine kinase of the RAF family which mediates downstream cellular responses to growth signals through the mitogen-activated protein kinase (MAPK) signaling pathway. Oncogenic mutations in the BRAF gene, 80% of which are a single V600E substitution within the kinase domain, constitutively activate the MAPK signaling pathway and result in increased cell proliferation and apoptosis resistance. The V600E mutation is observed in colorectal cancer, non-Hodgkin lymphoma, papillary thyroid carcinoma, malignant melanoma, non-small-cell lung carcinoma, and lung adenocarcinoma. BRAF V600E is therefore an important immunohistochemical marker for tumor diagnosis and prognosis.

3. Principles and Procedures

Visualization of the antigen present in tissue sections is accomplished in a multi-step immunohistochemical staining process, in conjunction with a horseradish peroxidase (HRP) or alkaline phosphatase (AP) linked detection system. The process involves the addition of the stated antibody (primary antibody) to a tissue slide, followed by a secondary antibody (link antibody) which specifically binds to the primary antibody. A chromogenic substrate is then added which reacts with the enzyme complex, resulting in a colorimetric reaction at the site of the antigen. Results are interpreted using a light microscope.

Product Information

REF

Description

IHC600-100	0.1 ml Concentrate
IHC600-1	1.0 ml Concentrate
IHC600-7	7.0 ml Pre-dilute, Ready-to-Use
IHC600-PC	Positive control slides, 3 slides/case

1 - 604 - 244 - 9962
info@GenomeMe.ca
www.GenomeMe.ca



Distributed By:
IBL-America, Inc.
8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA
info@ibl-america.com
(888) 523 1246



4. Materials and Methods

Reagents Provided

Product	Optimized Buffer Composition
Predilute	Antibody Diluent Buffer
Concentrate	Tris Buffer, pH 7.3 - 7.7, with 1% BSA and <0.1% Sodium Azide
Recommended working dilution range	1:100 - 1:200

Reconstitution, Mixing, Dilution, and Titration

The prediluted antibody does not require any mixing, dilution, reconstitution, or titration; the antibody is ready-to-use and optimized for staining.

The concentrated antibody requires dilution in the optimized buffer, to the recommended working dilution range (see Reagents Provided).

Storage and Handling

Store at 2-8°C. Do not freeze.

When stored correctly, the antibody is stable until the date indicated on the label.

To ensure proper stability and delivery of the antibody after each run, replace the cap and immediately place the bottle in a refrigerator in an upright position.

Positive and negative controls should be simultaneously run with unknown specimens, as there are no conclusive characteristics to suggest instability of the antibody. If such an indication of instability is suspected, contact GenomeMe® Customer Service at info@GenomeMe.ca.

Specimen Collection and Preparation for Analysis

Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4µm), and placed on a glass slide that is positively charged. The prepared slide may then be baked for a minimum of 30 minutes in a 53-65°C oven (do not exceed 24 hours).

Note: Performance evaluation has been shown on human tissues only. Variable results may occur due to extended fixation time or special processes of specific tissue preparations.

5. Instructions For Use

Recommended Staining Protocols for the BRAF V600E (IHC600) antibody:

Manual Use:

- Pretreatment:** Perform heat-induced epitope retrieval (HIER) at pH 9 for 10 to 30 minutes.
- Peroxide Block:** Block in peroxidase blocking solution for 5 minutes at room temperature. (Not required if using Alkaline Phosphatase System)
- Primary Antibody:** Apply antibody directly (Pre-dilute) or dilute antibody 1:100-1:200 (Concentrate) before applying. Incubate antibody for 10 to 30 minutes at room temperature.
- Secondary Antibody:** Incubate for 20 to 30 minutes at room temperature.
- Substrate Development:** Incubate DAB or Fast Red for 5 to 10 minutes at room temperature.
- Counterstain:** Counterstain with hematoxylin for 0.5 to 5 minutes, depending on the hematoxylin used. Rinse with distilled water and blueing solution for 30 seconds.
- Dehydrate and apply coverslip.

Automated Staining System:

The stated primary antibody has been validated using Leica® Biosystems' BOND-MAX Autostainer, applying IHC Protocol F. The following edits are recommended for the protocol:

- Marker Incubation Time: 30 Minutes
- Heat-induced epitope retrieval (HIER) is recommended using Bond ER Solution 2 for 30 minutes.
- Move Peroxide Block step to after Polymer and before Mixed DAB refine

For all other automated IHC staining systems, please refer to the corresponding user manual for specific instructions.

6. Quality Control Procedures and Interpretation of Results

The immunohistochemical staining process results in a colorimetric reaction at the site of the antigen, localized by the primary antibody.



Positive Control Tissue

A positive control tissue must be run with each staining procedure, and must be prepared and fixed identically to the test sections in order to provide control for all test variables, including fixation and tissue processing. The positive control tissue should be fresh autopsy, biopsy, or surgical specimens. For optimal quality control and to allow detection of lesser levels of reagent degradation, a tissue with weaker positive staining is advisable. Colorectal adenocarcinoma tissue can be used as positive control tissue for the BRAF V600E (IHC600) antibody. Where applicable, tissue that contains cells or tissue components that stain both positively and negatively may serve as both the positive and negative control tissue.

Once stained, the positive control tissue should be analyzed to ensure appropriate positive staining is observed and all reagents are functioning properly. Positive reactivity requires the observation of an appropriate colorimetric reaction at the site of the antigen within the target cells. Counterstaining will result in a blue coloration, which may be pale to dark depending on the length of the incubation time and potency of the hematoxylin.

If positive staining as defined herein is not observed, the results obtained must be treated as invalid. The positive control tissue should be used solely as a measure of performance of the reagents and validity of obtained results.

Negative Control Tissue

The same tissue used for the positive control tissue may be used as the negative control tissue.

Most tissue sections offer internal negative control sites due to the diversity of cell types present, however this must be confirmed by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non-specific background staining. If specific staining is observed, the negative control tissue must be deemed invalid and the results obtained must also be treated as such.

Tissue Specimens

Tissue specimens should be analyzed only once the positive and negative control tissues have been deemed as valid. Negative staining indicates that the antigen was not detected; the use of a panel of antibodies may allow for recognition of false negative results, as negative staining in any one test does not confirm the absence of the antigen in question.

7. Troubleshooting

1. If the tissue sections wash off the slide, this may be due to:
 - a) If the slides are not positively charged
 - b) Inadequate drying of the tissue section prior to staining
 - c) Inadequate neutral-buffering of the formalin used for the fixation process
 - d) The thickness of the tissue
2. If the positive control tissue exhibits negative staining, this may be due to:
 - a) The primary antibody or one of the secondary reagents.
 - b) Improper collection, fixation, or deparaffinization of the tissue section.
3. If the positive control tissue exhibits weaker staining than expected, this may be due to the primary antibody or one of the secondary reagents. Any other positive controls run simultaneously should be analyzed to determine the cause.
4. If non-specific staining occurs, this will have a diffuse appearance and may be due to:
 - a) Improper or suboptimal fixation of tissue sections which may result in sporadic light staining of connective tissue.
 - b) The use of necrotic or degenerated cells. Intact cells should be used for analysis of staining results.

For assistance with all other inquiries, contact GenomeMe® Customer Service at info@GenomeMe.ca.

8. Limitations

1. Due to inevitable variability in immunohistochemical procedures and variables, appropriate positive and negative controls must be used and documented.
2. Improper handling and processing of tissue samples may compromise the validity and/or analysis of the results.
3. GenomeMe® provides prediluted antibodies in a ready-to-use, optimally diluted format for use explicitly



as instructed. Improper handling and processing of tissue samples and reagents, and any deviation from the recommended procedures outlined herein, may compromise the validity and/or analysis of the results. Due to the potential for variation in tissue processing and fixation, it may be necessary to adjust the incubation time for the primary antibody on specific tissue specimens.

4. GenomeMe® provides concentrated antibodies in a format that requires dilution in the optimized buffer, in the context of appropriate validation by the user. Any diluent different than that specified in the package insert must also be validated by the user to ensure proper compatibility with the antibody. Once diluted, any deviation from the recommended procedures outlined herein may compromise the validity and/or analysis of the results.
5. This antibody, when used with the appropriate detection systems and accessories, detects antigen(s) that remain intact through the tissue fixation, processing, and sectioning as described herein. Any deviation from these recommended procedures may compromise the validity and/or analysis of the results.
6. Any documented discrepancies or unexplainable results in controls or tissue specimens should be reported to GenomeMe® Customer Service at info@GenomeMe.ca. Results are invalid if analysis of the positive and negative control tissues yields results other than those approved and described herein. The Troubleshooting section of this insert may be referred to for unexplained discrepancies in control tissues.
7. The potential for unexpected results cannot be eliminated due to inherent biological variability in the expression of certain antigens.
8. The potential for false positive results cannot be eliminated due to the possibility of non-immunological binding of substrate reaction products or proteins. False positive results may also occur subject to the type of immunostaining technique used, or due to the activity of pseudo-peroxidase, endogenous peroxidase, or endogenous biotin.
9. Due to the effect of autoantibodies or natural antibodies, normal sera from an animal source the same as the secondary antisera may result in false negative or false positive results when used in blocking steps.

9. Warnings and Precautions

1. Ensure proper handling procedures are used with all reagents. Always wear laboratory coats, disposable gloves, and other appropriate laboratory equipment when handling reagents.
2. Do not ingest reagents, and avoid contact with eyes and mucous membranes. Wash eyes with copious amounts of water if contact occurs.
3. All incubation times and temperatures must be validated by the user, as must any storage conditions different than those specified in the package insert.
4. Prediluted antibody is provided in a ready-to-use, optimally diluted format, and any further dilution may result in loss of antigen staining.
5. Concentrated antibody requires dilution in the optimized buffer (refer to *Reagents Provided*), in the context of appropriate validation by the user.
6. Handle tissue sections and all materials contacting them as biohazardous materials, using the appropriate precautions.
7. To ensure proper stability of the antibody and validity of results, use proper handling of the reagent and avoid microbial contamination.

10. References

1. Li WQ, et al. *Mol Cancer*. 2006; 5:2
2. Davies H, et al. *Nature*. 2002; 417:949-954.
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4. Gear H, et al. *Invest Ophthalmol Vis Sci*. 2006; 45:2484-8.
5. Capper D, et al. *Acta Neuropathol*. 2011; 122:11-19.