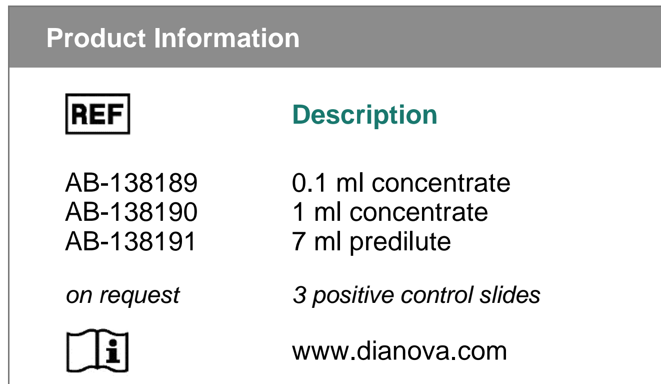
BRAF V600E

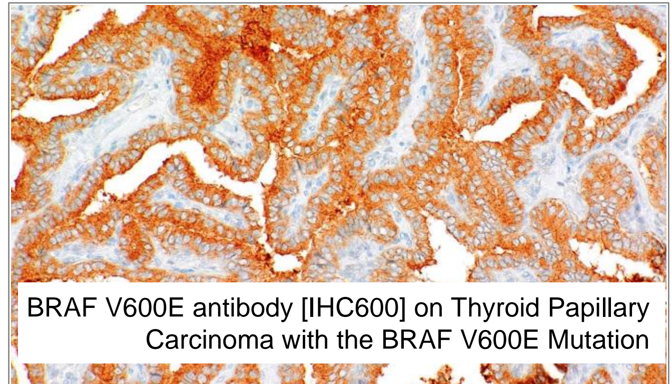
Mouse Monoclonal Antibody

Clone: IHC600
Host: Mouse
Isotype: N/A
Positive Control: Colorectal Adenocarcinoma, Thyroid Papillary Carcinoma with the BRAF V600E Mutation



Product Information

REF	Description
AB-138189	0.1 ml concentrate
AB-138190	1 ml concentrate
AB-138191	7 ml predilute
on request	*3 positive control slides*
i	www.dianova.com



BRAF V600E antibody [IHC600] on Thyroid Papillary Carcinoma with the BRAF V600E Mutation

1. Intended Use

This antibody is intended for *in vitro* diagnostic use.

The BRAF V600E 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. Use of this antibody is indicated, subsequent to clinical differential diagnoses of diseases, as an aid in the identification of mutant BRAF protein expression in diverse cancers within the context of antibody panels, the patient's clinical history and other diagnostic tests evaluated by a qualified pathologist.

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's lymphoma, papillary thyroid carcinoma, malignant melanoma, non-small-cell lung carcinoma, and lung adenocarcinoma. BRAF V600E is therefore an important immunohistochemical marker for tumour 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 (linked to an enzyme complex) 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.

4. Materials and Methods

Product Format	Optimized Buffer Composition
Predilute	Antibody Diluent Buffer
Concentrate	Tris Buffer, pH 7.3 - 7.7, with 1% BSA and <0.1% Sodium Azide

Note: The recommended **working dilution is 1:30**.

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. Any further dilution may affect the quality of the staining signal or antibody-antigen interaction.

The concentrated antibody requires dilution in the optimized buffer, to the recommended working dilution range mentioned above, prior to use.

Storage and Handling

Store at 2-8°C. Do not freeze.

To ensure stability, immediately replace vial back in the refrigerator after each use. When stored correctly, the antibody is stable until the expiry date indicated on the label.

Positive and negative controls should be concurrently run with tissue specimens, to enable identificationof any

inadequacies with the antibody or reagents. If antibody stability issues are suspected, please contact DIANOVA Technical Support at **info@dianova.de**.

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.

Material Required but not Provided

The following materials are required but are not provided:

a) Detection system (ie. BOND Polymer Refine Detection Kit or UltraView Universal DAB Detection Kit)
b) Chromogen (ie. DAB Substrate Kit)
c) IHC wash buff er and blocking solution
d) Hematoxylin or other counterstaining reagents
e) Ethanol or reagent alcohol, xylene or xylene substitute and mounting medium
f) Antibody diluents
g) Positive and negative control tissue

5. Instructions For Use

Recommended Staining Protocols for the BRAF V600E [IHC600] antibody:

Automated Staining with Leica Biosystems Bond-MAX Platform:

This primary antibody has been optimized and validated using the Leica Bond-MAX Fully Automated IHC & ISH Stainer, applying IHC Protocol F. Antibody concentrate dilution range is 1:30.

The following edits are recommended for the protocol:

a) Marker Incubation Time: 30 minutes
b) Heat-induced epitope retrieval (HIER) is recommended using Leica Bond ER Solution 2 for 30-40 minutes.
c) Move Peroxide Block step to after Polymer and before Mixed DAB Refine.

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

Automated Staining with Ventana BenchMark ULTRA Platform:

This primary antibody has been optimized and validated using the Ventana BenchMark ULTRA IHC/ISH System. Antibody concentrate dilution range is 1:30.

Recommended protocol parameters are as follows:

a) Detection Kit: OptiView DAB IHC
b) Pretreatment Protocol: CC1 64 minutes, 98-100°C
c) Primary Antibody: 16-20 minutes, 36°C

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

Manual Use:

1. **Blocking:** If HRP is used, block with peroxidase blocking solution for 10-15 minutes at room temperature. Replace with alkaline phosphatase blocking solution if an AP system is used.
2. **Primary Antibody**: Apply and incubate antibody for 30-60 minutes at room temperature or overnight at 4°C.
3. **Secondary Antibody:** Apply and incubate for 20 to 30 minutes at room temperature.
4. **Substrate Development:** Apply and incubate with DAB or Fast Red for 5 to 10 minutes at room temperature.
5. **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.
6. Dehydrate and apply coverslip.

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. A qualified pathologist must interpret the tissue specimen results only after the positive and negative control tissues have been analyzed. It is recommended to include a set of tissue controls with each staining run to monitor for antibody, tissue, and reagent performance.

Tissue sections may contain both positive and negative staining elements. In these cases and where applicable, these sections may serve as both the positive and negative tissue control.

Positive Control Tissue

A positive control tissue should be processed in the same manner as the specimen and run with each test condition to provide control for variables such as tissue processing, fixation, and staining. It should function to provide validity to the specimen results obtained and can consist of fresh autopsy, biopsy, or surgical tissue.

Once stained, the positive control tissue should analyzed first to ensure that the antibody and all reagents are performing as intended. Counterstaining will result in a blue coloration, which may range from pale to dark depending on the length of the incubation time and potency of the hematoxylin. If positive staining is not observed, the positive control tissue must be deemed invalid and the results obtained with the tissue

specimen must also be treated as such.

Negative Control Tissue

Some tissue sections can also function as an internal negative control due to the diversity of staining elements present. This, however, should first be confirmed by the user. Tissue components that do not stain should demonstrate an absence of specific staining. If specific staining is observed, the negative control tissue must be deemed invalid and the results obtained with the tissue specimen must also be treated as such.

Tissue Specimens

Tissue specimens should only be analyzed after the positive and negative control tissues have been deemed valid. Negative staining indicates that the antigen was not detected in the tissue while positive staining represents the presence of the antigen. A tissue section stained with hematoxylin and eosin should be used to analyze the morphology of the tissue specimen and verified by a qualified pathologist.

Tissue Specimens

This antibody has been validated by immunohistochemistry using a FFPE human tissue microarray comprised of different types of normal and cancerous tissues. Positive staining was observed on tissue from thyroid papillary carcinoma with BRAF V600E mutation. No staining was observed on tonsil, breast cancer, lung cancer and brain tissues. A representative positive staining image is shown on Page 1.

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) A thick tissue section.
2. If the positive control tissue exhibits negative staining, this may be due to:
   a) An issue with the primary antibody or one of the secondary reagents.
   b) Improper collection, fixation, or deparaffinization of the tissue section.
   c) Errors in the IHC staining process.
3. If the positive control tissue exhibits weaker staining than expected, this may be due to sub-optimal IHC conditions, partial degradation of the primary antibody or improper storage of secondary reagents. Analysis of the positive and/or negative control tissues can help with determining the cause.

For assistance with all other types of inquiries, contact DIANOVA Technical Support at **info@dianova.de**.

8. Limitations

1. This antibody is intended for in vitro diagnostic (IVD) use by qualified laboratories only and is not intended for use in flow cytometry.
2. Due to inevitable variability in immunohistochemical procedures and variables, appropriate positive and negative controls must be used and documented, and the results are to be interpreted by a qualified pathologist. Staining must be conducted in a certified, licensed laboratory, under the supervision and responsibility of the qualified pathologist.
3. Improper handling and processing of tissue samples may compromise the validity and/or analysis of the results.
4. DIANOVA 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 incubation time for the primary antibody on specific tissue specimens.
5. DIANOVA 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.
6. 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 deviations from these recommended procedures may compromised the validity and/or analysis of the results.
7. The clinical outcome indicated by staining results must be analyzed accurately by the qualified pathologist, and the patient's medical history and other histopathological criteria must be taken into account. The user is responsible for interpretation of the results in the context of the patient.
8. Any documented discrepancies or unexplainable

results in controls or tissue specimens should be reported to DIANOVA Technical Support at info@dianova.de. Patient 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.

9. The potential for unexpected results in patient tissue specimens cannot be eliminated due to inherent biological variability in the expression of certain antigens.
10. The potential for false positive results in patient tissue specimens 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 pseudoperoxidase, endogenous peroxidase, or endogenous biotin.
11. 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.
12. Non-specific staining with horseradish peroxidase may be observed when using tissues containing hepatitis B surface antigen due to the patient's infection with the hepatitis B virus.

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, patient specimens, 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.

Dianova GmbH
Warburgstrasse 45, 20354 Hamburg, Germany

10. References

1. Li WQ, et al. Mol Cancer. 2006; 5:2
2. Davies H, et al. Nature. 2002; 417:949-54
3. Benlloch S, et al. J Mol Diagn. 2006; 8:540-3
4. Gear H, et al. Invest Ophthalmol Vis Sci. 2006; 45:2484-8
5. Capper D, et al. Acta Neuropathol. 2011; 122:11-19.