

Melanoma

Mouse Monoclonal Primary Antibody

Format	Catalog No.	Pack size	Dilution
Concentrated	G0282 A, B, C	0.1 ml	1: 100
		0.5 ml	1: 200
		1.0 ml	
Prediluted	G0282 AA, BB	6.0 ml	RTU
		3.0 ml	

INTENDED USE

This antibody is intended for **Research Use Only (RUO)**. The purpose of the Melanoma Antibody Mouse Monoclonal Antibody is to identify the Melanoma protein in tissue that has been paraffin-embedded and fixed in formalin using manual qualitative immunohistochemistry (IHC) testing. After traditional histopathology has employed non-immunological histochemical stains to make the first tumour diagnosis, this antibody is meant to be used. A certified pathologist must interpret the results of this product as a diagnostic tool in combination with the patient's pertinent clinical history, additional diagnostic testing, and appropriate controls.

SUMMARY AND EXPLANATION

HMB45 is a widely recognized monoclonal antibody used to detect a specific melanosomal glycoprotein. It is highly reactive with a range of melanocytic lesions, especially melanoma. HMB45 typically labels junctional nevus cells and fetal melanocytes but does not react with normal adult melanocytes. It is a useful marker for active melanogenesis. Re-expression can occur in activated melanocytes under certain physiological conditions. HMB45 may occasionally show staining in hair follicles, but it generally does not label melanocytes in lentigines or benign fibroblastic proliferations. Its staining profile is highly selective and rarely observed in normal non-melanocytic tissues.

PRINCIPLE OF METHOD

Mouse Melanoma Antibody Sections that have been paraffin-embedded and formalin-fixed are employed with monoclonal antibodies. It is advised to pretreat deparaffinized tissue using either enzymatic or heat-induced epitope retrieval. Immunohistochemical (IHC) staining methods enable the visualisation of antigens through the sequential application of an enzyme complex,

a chromogenic substrate, a secondary antibody to the primary antibody (link antibody), and a specific antibody to the antigen (primary antibody), with washing steps in between. A visible reaction product is produced at the antigen site because of the chromogen's enzymatic activity. A light microscope is used to interpret the results, which help with the differential diagnosis of pathophysiological processes that might or might not be connected to a specific antigen.

MATERIALS PROVIDED

Melanoma Antibody Mouse Monoclonal in concentrated form or prediluted Antibody Specifications: Antibody as Purified diluted in Tris-buffer of Ph 7.4, containing a highly purified carrier protein and less than 0.1% sodium azide preservative.

- **HOST SPECIES:** Mouse
- **CLONE:** HMB45
- **ISOTYPE:** IgG1/kappa
- **CELLULAR LOCALIZATION:** Cytoplasm
- **IMMUNOGEN:** Developed using tissue extracts from pigmented melanoma metastases in lymph nodes of BALB/c mice.
- **MOLECULAR WEIGHT:** ~100 kDa
- **SPECIES REACTIVITY:** Human
- **POSITIVE CONTROLS:** Human melanoma tissue

STAINING PROCEDURE

The table below outlines the specific conditions recommended for this antibody.

Parameter	Genebio Solution Recommendations
Pretreatment	EDTA Buffer pH 9.0 (Manual/ AntigenXpress)
Incubation Time & Temperature	30-60 min @ RT
Tissue Type	FFPE

STORAGE AND HANDLING

Store the vial between 2 and 8°C after receiving it. This antibody is stable for 24 months when kept between 2 and 8°C. The bottle must be put in a refrigerator right away in an upright position when the cap is replaced to guarantee correct reagent stability and functionality. Never use after the vial's imprinted expiration date. The user must confirm



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whether reagents are stored in any other conditions than those listed in the package insert. Repeated cycles of freezing and thawing should be avoided since they may reduce antibody activity. Do not use and get in touch with Genebio if the contents or package seem damaged or broken. The final page of this paper contains contact details.

RECONSTITUTION

Antibodies that have been prediluted are ready to use; reconstitution is not required. Concentrate Antibodies: Use the proper lab-standardized diluent and container and refer to the established dilution range of 1:100 to 1:200. If there are any special instructions, see the label. According to stability tests conducted at Genebio using polypropylene containers, stability after dilution is 7 days at 24°C, 3 months at 2–8°C, and 6 months at -20°C.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Positive Tissue Control
2. Negative control tissue (internal or external)
3. Microscope slides and coverslips
4. Staining jars or baths
5. Timer
6. Xylene or xylene substitute
7. Ethanol or reagent alcohol
8. Deionized or distilled water
9. Heating equipment or enzyme for tissue pretreatment step
10. Detection system
11. Chromogen
12. Wash Buffer
13. Hematoxylin
14. Antibody diluents
15. Peroxide Block
16. Light Microscope
17. Mounting medium
18. Avidin-Biotin Blocking Reagents for use with streptavidin biotin detection

SPECIMEN PREPARATION FOR ANALYSIS

This primary antibody is utilised with neutral-buffered, paraffin-embedded, formalin-fixed tissue sections that are routinely processed. Tissue sections of around 4µm are ideal, and they ought to be put on positively charged slides.

It is advised to pretreat deparaffinized tissue with heat-induced epitope retrieval. Long-term fixation or the unanticipated addition of extraneous objects or interfering substances may cause different results. The staining findings may also be impacted by decalcification. Horseradish peroxidase may stain tissues from hepatitis B virus-infected individuals that carry hepatitis B surface antigen (HBsAg) non-specifically. Horseradish peroxidase may stain tissues from hepatitis B virus-infected individuals that carry hepatitis B surface antigen (HBsAg) non-specifically.

WARNINGS AND PRECAUTIONS

1. When working with chemicals, exercise reasonable caution. When working with dangerous substances (like xylene) or probable carcinogens, wear lab coats and disposable gloves.
2. Keep reagents away from mucous membranes and eyes. Wash thoroughly with water if reagents meet delicate parts.
3. All things that come into touch with patient specimens should be handled as biohazardous materials and disposed of carefully. Never use your mouth to pipette.
4. The user needs to confirm the temperatures and incubation periods.
5. The ready-to-use, prediluted reagents are already optimally diluted; additional dilution could cause the antigen staining to disappear.
6. Depending on user validation, the concentrated reagents may be optimally diluted. The user must also confirm the compatibility and stability impact of any diluent employed.
7. This product is not considered a hazardous material when used as directed. The reagent's preservative, which is less than 0.1% ProClin, does not, at the specified concentration, satisfy OSHA's (USA) requirements for hazardous substances. Consult Genebio Solution's Safety Data Sheet (SDS) online.
8. Any storage conditions that differ from those listed in the package insert must be verified by the user.
9. Bovine serum albumin and serum may be present in the diluent and supernatant, respectively. Commercial suppliers are the source of the products containing bovine serum albumin and foetal bovine serum. According to the certificates, the bovine origins are from the United States and Canada and come from nations with very low BSE risk.
10. Use the right handling techniques, just like you would with any product made from biological sources.



QUALITY CONTROL PROCEDURES

Positive Tissue Control

Every staining method must be followed by a positive tissue control. This tissue can function as both the positive and negative control tissue and may have both positive and negative staining cells or tissue components. Fresh autopsy, biopsy, or surgical specimens should be used as external positive control materials. They should be preserved, processed, and embedded as quickly as feasible, using the same protocol as the patient sample or samples. Proper staining techniques and properly prepared tissues are shown by positive tissue controls. Patient specimens with well-characterized low levels of the positive target activity that result in weak positive staining should be used to choose the tissues for the external positive control materials. For external positive controls, the low degree of positivity is intended to guarantee the detection of minute variations in the main antibody sensitivity caused by instability or issues with the staining technique. The best quality control and detection of trace amounts of reagent degradation are achieved with a tissue that exhibits faint positive staining.

Negative Control Tissue

Depending on the rules and regulations governing the organisation the end user is a part of, either internal or external negative control tissue may be utilised. Internal negative control sites are provided by the diversity of cell types found in numerous tissue sections; nonetheless, the user should confirm this. The non-staining components should indicate non-specific background staining and show that particular staining is absent. Results involving patient specimens must be deemed invalid if specific staining takes place in the negative tissue control sites.

Patient Tissue

It is best to analyse patient specimens last. Any nonspecific background staining of the negative reagent control should be considered when evaluating the strength of positive staining. A negative result indicates that the antigen was not found, not that it was not present in the cells or tissue examined, as is the case with all immunohistochemistry tests. Use an antibody panel if required to detect false-negative results.

INTERPRETATION OF RESULTS

A coloured reaction product precipitates at the antigen locations that the primary antibody has localised as a result of the immunostaining process. For information on expected colour reactions, consult the relevant detection system package insert. Before interpreting the data, the control tissues must be assessed by a certified pathologist skilled in immunohistochemical techniques.

Positive Tissue Control

To make sure that all of the reagents are working correctly, the stained positive control should be inspected first. Positive reactivity is demonstrated when the target cells or markers have a reaction product of the proper colour. Consult the product insert for the colour response detection mechanism. Inadequate or excessive staining can make it more difficult to properly interpret the data. Any results involving the specimens are deemed invalid if the positive tissue control does not exhibit the proper positive staining.

Negative Tissue Control

To confirm the precise labelling of the target antigen by the main antibody, the negative tissue control (internal or exterior) should be analysed following the positive tissue control. The lack of antibody cross-reactivity to cells or cellular components is confirmed by the absence of particular staining in the negative tissue control. The patient specimen's results are deemed invalid if specific staining is detected in the negative tissue control.

Patient Tissue

The following step should be to evaluate the patient's tissues. Any background staining of the (internal) negative control should be considered when evaluating positive staining. A negative result indicates that the antigen was not found, not that it is not present in the cells or tissue being examined, as is the case with all immunohistochemistry tests. False negative reactions can be identified with the help of an antibody panel. A trained pathologist must interpret the patient's morphologic results and pertinent clinical data.

LIMITATIONS

1. Because immunohistochemistry is a multi-step process that necessitates specific training in the selection of the right reagents, tissues, fixation, and processing; the preparation of the immunohistochemistry slide; and the



interpretation of the staining results, this reagent is only for professional use in the laboratory.

2. The way the tissue is handled and processed before staining affects the staining process. Artefacts, antibody trapping, or false negative results can result from improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids. Variations in fixation and embedding techniques, as well as intrinsic abnormalities in the tissue, might lead to inconsistent outcomes.

3. Inadequate or excessive counterstaining could make it more difficult to properly interpret the data.

4. Any positive staining—or lack thereof—must be clinically interpreted considering the patient's medical history, morphology, other histopathological standards, and other diagnostic procedures. If appropriate, this antibody is meant to be employed in a panel of antibodies. A competent pathologist must be knowledgeable about the diagnostic panels, chemicals, antibodies, and techniques utilised to create the stained preparation. Under the guidance of a pathologist who oversees examining the stained slides and guaranteeing the sufficiency of positive and negative controls, staining must be carried out in a laboratory with a licence and certification.

5. Genebio offers antibodies at the ideal dilution for use in both concentrated and prediluted forms. Please see pages 1 and 2 for Dilution Range and Reconstitution. Any departure from the suggested test protocols could render the anticipated outcomes invalid. It is necessary to implement and record appropriate controls. In any case, users are accountable for how they interpret patient results.

6. Genebio offers certain antibodies in concentrated form, which the user can then dilute to the ideal concentration for use, provided that the user determines and follows appropriate validation procedures. Any diluents used beyond those suggested here (see Dilution Range) must be verified by the user. In any case, users are accountable for how they interpret patient results.

8. Reagents may react unexpectedly in tissues that haven't been evaluated before. Because of the biological diversity of antigen expression in neoplasms or other diseased tissues, it is impossible to totally rule out the possibility of unexpected reactions, even in studied tissue groups.

8. Hepatitis B surface antigen (HBsAg)-containing tissues from hepatitis B virus-infected individuals may show nonspecific staining with horseradish peroxidase.

9. Due to the influence of autoantibodies or natural antibodies, normal sera from the same animal source as the secondary antisera may provide false positive or false negative results when employed in blocking phases.

10. Nonimmunological binding of proteins or substrate reaction products may result in false positive results. Depending on the kind of immunostaining method employed, they could also be brought on by endogenous biotin (liver, brain, breast, kidney), endogenous peroxidase activity (cytochrome C), or pseudoperoxidase activity (erythrocytes).

11. A negative result indicates that the antigen was not found, not that it was not present in the cells or tissue examined, as is the case with all immunohistochemical tests.

12. The ready-to-use prediluted antibody products are optimised. The primary antibody incubation period on individual specimens may need to be adjusted due to the potential for variation in tissue fixation and processing.

13. This antibody identifies antigen(s) that withstand standard tissue processing, sectioning, and formalin fixation when used in conjunction with detection systems and accessories. Users are still in charge of interpreting and validating patient results if they depart from the suggested test techniques here, just as they would in any other situation.

14. Only for use in laboratories.

Since every antibody has a distinct intended use, property, and effect, a qualified pathologist should evaluate and interpret staining data accordingly.

