

Mouse anti-Human Her2/Neu Monoclonal Antibody | anti-Her2/Neu antibody

Her2/Neu

CATALOG # AAA58780	HOST Mouse	REACTIVITY Human
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PRODUCT SYNONYMS

Her2/Neu; Monoclonal Antibody; anti-Her2/Neu antibody

PRODUCT SPECIFICATIONS

Host	Mouse
Reactivity	Human
Clonality	Monoclonal
Positive Control	Breast Carcinoma
1. Intended Use	The HER2/neu 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	The HER2/neu (c-erbB-2) proto-oncogene is a transmembrane receptor tyrosine kinase that is clinically indicated in a number of carcinomas. Overexpression of the c-erbB-2 protein has been associated with ductal breast cancer, as well as pulmonary and gastric adenocarcinomas. A correlation between HER2 and p53 has also been documented, as overexpression of both proteins has been associated with early invasion and metastasis in bladder cancer.
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: Predilute Optimized Buffer Composition: Antibody Diluent Buffer Product Format: Concentrate Optimized Buffer Composition: Tris Buffer, pH 7.3 - 7.7, with 1% BSA and <0.1% Sodium Azide Note: The recommended working dilution range is 1:50-1:200. 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 table above). Specimen Collection and Preparation for Analysis: Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4um), 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	This protocol is a recommendation only. Each researcher should optimize the protocol for their own use: Manual Use: 1. Pretreatment: Perform heat-induced epitope retrieval (HIER) at pH 9 for 10 to 30 minutes. 2. Peroxide Block: Block in peroxidase blocking solution for 5 minutes at room temperature. (Not required if using Alkaline Phosphatase System.) 3. Primary Antibody: Apply antibody directly (Predilute) or dilute antibody at 1:100-1:400 (Concentrate) before applying. Incubate antibody for 60 minutes at room temperature. 4. Secondary Antibody: Incubate for 20 to 30 minutes at room temperature. 5. Substrate Development: Incubate DAB or Fast Red for 5 to 10 minutes at room temperature. 6. 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. 7. Dehydrate and apply coverslip. Automated Staining System: The stated primary antibody has been optimized and validated using the BOND-MAX fully automated IHC & ISH stainer manufactured by Leica Biosystems, applying IHC Protocol F. The following edits are recommended for the protocol: a) Marker Incubation Time: 60 minutes b) Heat-induced epitope retrieval (HIER) is recommended using Leica Bond ER Solution 2 for 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.
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 scientific professional must interpret results only once the positive and negative control tissues have been analyzed. 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. Breast carcinoma tissue can be used as positive control tissue for the HER2/neu 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 with the tissue specimen must be treated as invalid. The positive control tissue should not be used as an aid for interpreting samples, but rather 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 with the tissue specimen must also be treated as such. Tissues Specimens 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. A tissue section stained with hematoxylin and eosin should be used to analyze the morphology of the tissue sample, as verified by a qualified scientific professional.

7. Troubleshooting	- If the tissue sections wash off the slide, this may be due to: - If the slides are not positively charged. - Inadequate drying of the tissue section prior to staining. - Inadequate neutral-buffering of the formalin used for the fixation process. - A thick tissue section. - If the positive control tissue exhibits negative staining, this may be due to: - The primary antibody or one of the secondary reagents. - Improper collection, fixation, or deparaffinization of the tissue section. - 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. - If non-specific staining occurs, this will have a diffuse appearance and may be due to: - Improper or suboptimal fixation of tissue sections which may result in sporadic light staining of connective tissue. - The use of necrotic or degenerated cells. Intact cells should be used for analysis of staining results.
8. Limitations	- This antibody is intended for Research Used Only by qualified laboratories only and is not intended for use in flow cytometry. - 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 scientific professionals. - Improper handling and processing of tissue samples may compromise the validity and/or analysis of the results. - Provided 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. - Provided 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. - 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. - The outcome indicated by staining results must be analyzed accurately by the scientific professional, and the other information such as, histopathological criteria must be taken into account. The researcher is responsible for interpretation of the results in the context all of known information about the sample and the donor. - Any documented discrepancies or unexplainable results in controls or tissue specimens should be evaluated by the researcher. Sample results may be invalid if analysis of the positive and negative control tissues yields results other than those described herein. The Troubleshooting section may be referred to for unexplained discrepancies in control tissues. - Any documented discrepancies or unexplainable results in controls or tissue specimens should be reported. - The potential for unexpected results in tissue specimens cannot be eliminated due to inherent biological variability in the expression of certain antigens. - The potential for false positive results in tissue specimens cannot be eliminated due to the possibility of nonimmunological 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. - 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. - Non-specific staining with horseradish peroxidase may be observed when using tissues containing hepatitis B surface antigen due to a donor's infection with the hepatitis B virus.

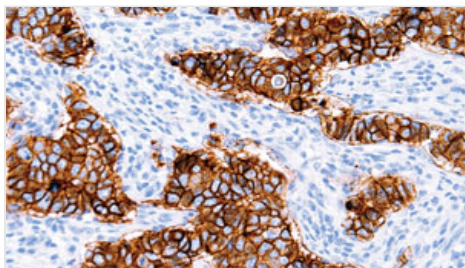
9. Warnings and Precautions

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, donor's 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.

Preparation and Storage

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.

IMAGES



IHC (Immunohistochemistry)
Her2/Neu on Breast

ADDITIONAL INFORMATION

Related Product Information for anti-Her2/Neu antibody

The Her2/Neu (c-erbB-2) proto-oncogene is a transmembrane receptor tyrosine kinase that is clinically indicated in a number of carcinomas. Overexpression of the c-erbB-2 protein has been associated with ductal breast cancer, as well as pulmonary and gastric adenocarcinomas. A correlation between Her2 and p53 has also been documented, as overexpression of both proteins has been associated with early invasion and metastasis in bladder cancer.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/monoclonal/her2-neu-antibody-elisa/58780>