Recombinant Monoclonal Anti-HER2 Antibody, Rabbit (5D5), Ready-To-Use

Catalog Number: CAA-B004

Specification: 7.5 mL

IMPORTANT: Please carefully read this user guide before performing your experiment

Product Information

This reagent can be used for immunohistochemical staining on the basis of conventional staining (such as H&E staining) to provide auxiliary information for laboratory tissue detection.

This reagent is used to detect the expression of cell HER2 on tumor cell membrane by immunohistochemical method. The antibody of HER2 specifically binds to the protein of HER2 expressed on the tissue, and the enzymo-labeled antibody is added to make the enzymo labeled antibody specifically bind to the antibody of HER2. The horseradish peroxidase labeled on the enzymo-labeled antibody catalyzes the DAB color development solution subsequently added to oxidize benzidine into biphenylimide. In this way, the antigen epitope in the tissue section appears brownish yellow or yellowish brown color, and finally the sample is re-stained and sealed. The presence site and expression of HER2 on the tissue section were deduced by observing the color development under microscope.

Precautions:

1. Do not use this product after the expiration date indicated on the reagent label.
2. Do not mix or substitute reagents with those from different brands or sources.
3. This reagent is for research use only, not for diagnostic or therapeutic application.

Contact Information:

Manufactured and distributed by
ACRODiagnostics Inc.

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Content

Recombinant Monoclonal Anti-HER2 Antibody, Rabbit (5D5) with antibody dilutent.

Storage

Stable for 12 months from the date of manufacture if stored at 2°C to 8°C, improper storage conditions will lead to test invalidation.

Application Platform

LEICA BOND III

Required Materials (Not Supplied)

Instrument	LEICA BOND-III Fully Automated IHC and ISH Stainer
Reagents	BOND Epitope Retrieval Solution 2
	BOND Wash Solution 10X
	BOND Polymer Refine Detection DAB
	Deionized Water
Consumables	Microscope Slides
	Coverslips
	Permanent mounting medium and ancillary reagents required for mounting coverslips.

Tissue Sample Requirements

All tissue sample slides must be prepared and properly preserved for immunohistochemistry assay with standardized processes. The suggestion is to prepare samples with formalin and do regular paraffin embedding.

For instance, tissue samples should be prepared as 3-4mm thick sections, and fixed into 10% neutral buffered formalin at room temperature for 18-24 hours. Followingly, sample slides are dehydrated through a graded alcohol series (e.g., 70% to 100% ethanol), transparentized with xylene, then infiltrated with molten paraffin wax at a temperature below 60°C for embedding.

Tissue samples should be cut into 3-5μm thick and mounted on the glass slides. The slides for processed HER2 protein evaluation and tumor validation should be made simultaneously and stored at 2–8°C (prepared) or room temperature (≤25°C), avoid light until use.

Detection Method

Pre-experiment Notice

1. Before immunostaining, make true CAA-B004 and other required reagents are all equilibrated to room temperature (18-27℃).
2. All incubation processes should be performed at room temperature (18-27℃).
3. Avoid slide drying during the staining procedures; otherwise, it may lead to increased non-specific background staining.

Reagent Preparation

BOND Wash Solution (1x washing buffer): Dilute 10x BOND Wash Solution Concentrate with 1:10 ratio to make sufficient BOND Wash Solution (1x washing buffer) with deionized water for IHC assay.

Operation Steps

1. **Dewaxing, Hydration and Antigen Retrieval**:
    a) Tissue sections are placed in a 62°C baking machine or oven for 30 minutes, and placed in xylene I and xylene II for 10 minutes each;
    b) Put in absolute ethanol I and absolute ethanol II for 5 minutes in order, put in 95% ethanol, 85% ethanol and 75% ethanol for 2 minutes in order, wash with pure water for 3 minutes × 3 times;
    c) Tissue section slides (FFPE) are immersed into BOND Epitope Retrieval Solution 2 (1x antigen retrieval solution). After incubate at 100℃ for 25 minutes, let tissue section slides cool down naturally to room temperature within the antigen retrieval solution. Wash the slides 5 times with BOND Wash Solution (1x washing buffer), then incubate at room temperature for 3 minutes.
2. **Blocking**: Incubate with inactivated endogenous peroxidase (Peroxide Block) at room temperature for 5 minutes and wash 3 times with BOND Wash Solution (1x washing buffer).
3. **Primary Antibody Incubation:** Add about 150 μL HER2 antibody reagent to each slide and incubate at

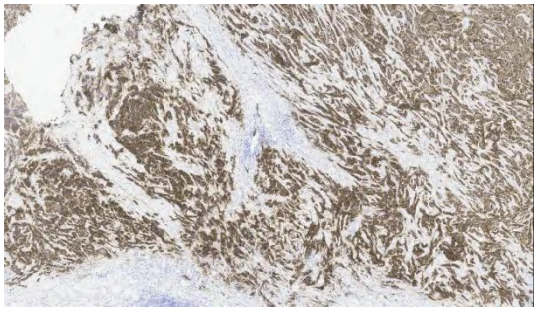
room temperature for 35 minutes. Then wash the slides 3 times with BOND Wash Solution (1x washing buffer).

4. **Secondary Antibody Incubation:**
   a) Add about 150 µL Post Primary (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 8 minutes, then wash the slides 2 minutes for 3 times with BOND Wash Solution (1x washing buffer).
   b) Add about 150 µL Polymer (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 8 minutes, then wash the slides 2 minutes for 2 times with BOND Wash Solution (1x washing buffer), 2 minutes for once with deionized water.
5. **DAB Color Development**: Add about 150 µL Mixed DAB Refine (BOND Polymer Refine Detection DAB) to each slide and incubate at room temperature for 10 minutes, then wash the slides 3 times with deionized water.
6. **Counterstaining:** Stain each slide with about 150 µL hematoxylin at room temperature for 5 minutes, then wash the slides once with deionized water, once with BOND Wash Solution (1x washing buffer), then once with deionized water.
7. **Mounting**: After dehydration and transparency, use an appropriate amount of mounting medium to mount, observe the slides under a microscope and interpret the results.

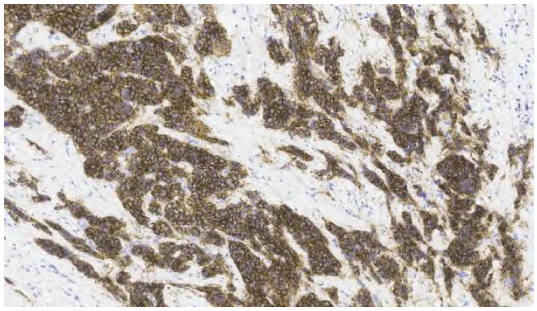
Typical Data

Control Sample & Cross-Reactivity

Staining of breast cancer tissue with the HER2 ready-to-use antibody showed positive staining on the cell membranes of breast cancer cells.



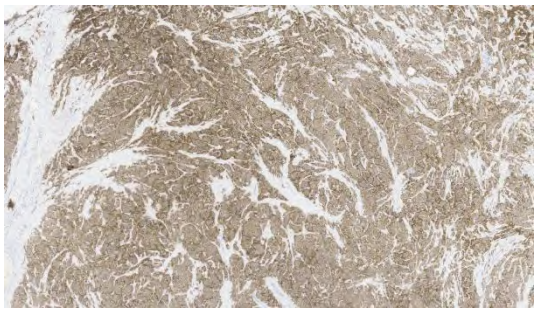
Breast cancer 4X



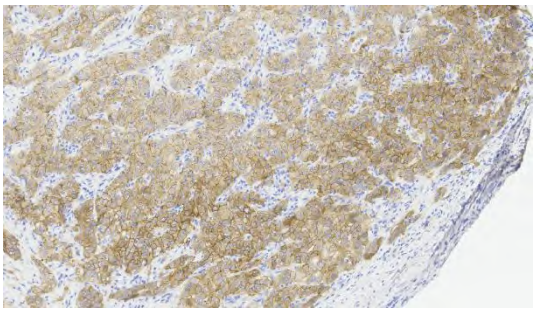
Breast cancer 20X

Cancer Sample & Indication Validation

Use CAA-B004 to stain breast cancer samples. The results are showed below:



Breast cancer 4X



Breast cancer 20X