

# HER2/neu [IHC042]

Concentrated and Prediluted Monoclonal Antibody  
902-3274-050522

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 3274 A, B	0.1, 0.5 mL	1:100	Renoir Red
Predilute	APR 3274 AA	6.0 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVR 3274 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALR 3274 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For Research Use Only. Not for use in diagnostic procedures.

## Summary and Explanation:

This antibody recognizes a protein of 185 kDa, identified as the second member (c-erbB-2/HER-2) of the c-erbB family (1,2). This rabbit monoclonal antibody is directed against the cytoplasmic domain of the human c-erbB-2 protein. The c-erbB-2 is closely related in structure to the epidermal growth factor receptor (2). The c-erbB-2 protein is overexpressed in a variety of carcinomas, especially those of breast and ovary and immunohistochemical staining correlates with gene amplification (1,2). Studies have shown that c-erbB-2 positive breast cancer usually correlates with negative staining for estrogen and progesterone receptors; thus, a poorer predictive outcome is correlated with c-erbB-2 staining (1,2).

## Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** IHC042

**Isotype:** IgG2a

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** HER2/neu

**Cellular Localization:** Membrane

**Positive Tissue Control:** Breast carcinoma known to be HER2 positive

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

## Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

## Staining Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidase 1.

**Pretreatment Solution:** Diva

## Pretreatment Protocol:

Heat Retrieval Method:

Preheat the retrieval solution to 95°C for 30 minutes and then place slides in the preheated solution if using Decloaking Chamber Pro or Decloaking Chamber Plus. If using Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 95°C for 40 minutes. Allow solution to cool for 20 minutes and then wash in distilled water.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** Incubate for 10 minutes at RT with a secondary polymer.

**Polymer:** Incubate for 20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

## Staining Protocol Recommendations (intelliPATH FLX® and manual use) Cont'd:

**Counterstain:** Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

## Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

## Staining Protocol Recommendations (Ventana BenchMark ULTRA):

AVR3274 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

**Template/Detection:** OptiView DAB IHC

**Pretreatment Protocol:** CC1 32 minutes

**Peroxidase:** Pre-Primary Peroxidase Inhibitor

**Primary Antibody:** 16 minutes, 36°C

## Staining Protocol Recommendations (Q Series – For Leica BOND-III):

ALR3274 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

**Protocol Name:** IHC protocol F

**Detection:** Bond Polymer Refine

**HIER:** 20 min with ER1

**Peroxide Block:** 5 min

**Marker (Primary Antibody):** 15 min

**Post Primary:** 8 min

**Polymer:** 8 min

**Mixed DAB Refine:** 10 min

**Hematoxylin:** 5 min

## Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

## Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (3)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (4)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

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### Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

### References:

1. Suthipintawong C, et al. Immunostaining of estrogen receptor, progesterone receptor, MIB1 antigen, and c-erbB-2 oncoprotein in cytologic specimens: a simplified method with formalin fixation. *Diagn Cytopathol.* 1997 Aug; 17 (2):127-33.
2. Nakapoulou LL, et al. Prognostic significance of the co-expression of p53 and cerbB-2 protein in breast cancer. *J Pathol.* 1996 May; 179(1):31-8.
3. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
4. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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