

c-erbB-2/HER2

Concentrated and Prediluted Rabbit Monoclonal Antibody
902-342-050522

BIOCARE
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CME 342 A, B	0.1, 0.5 mL	1:50	Da Vinci Green
Predilute	PME 342 AA, H	6.0, 25 mL	Ready-to-use	N/A
ONCORE	OAR 342 T60	60 tests	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALR 342 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. 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. The c-erbB-2 protein is overexpressed in a variety of carcinomas, especially those of breast and ovary. Immunohistochemical staining correlates with gene amplification. 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.

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: Rabbit monoclonal

Species Reactivity: Human

Clone: EP3 (previously known as EP1045Y)

Isotype: IgG

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: c-erbB-2 protein

Cellular Localization: Cell

membrane **Positive Control:** Breast

cancer **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 and manual use):

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized

1. Pretreatment Solution (recommended): Diva or Reveal

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 Biocare's Decloaking Chamber Pro or Decloaking Chamber Plus. If using Biocare's Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 95°C for 40 minutes. Allow solution to cool for

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

20 minutes then wash in distilled water.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30-45 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a secondary-conjugated polymer.

Chromogen:

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

Counterstain:

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

Technical Note:

This antibody has been optimized for use with Biocare's MACH 4 Universal HRP-Polymer Detection. Use TBS for washing steps.

Protocol Recommendations (ONCORE Automated Slide Staining System):

OAR342 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

Protocol Name: c-erbB-2 Rb

Protocol Template (Description): Rb HRP Template 1

Dewaxing (DS Option): DS Buffer

Antigen Retrieval (AR Option): AR2, low pH; 95°C

Reagent Name, Time, Temp.: c-erbB-2 Rb, 30 min., 25°C

Protocol Recommendations (O Series – For Leica BOND-III):

ALR342 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: 40 min with ER2

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.



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Rev: 062117

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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 (NaN₃) 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>.

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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