

## GCDFP-15 + Mammaglobin

Prediluted Multiplex Cocktail (4-Step)

Control Number: 901-317DS-083117

**Catalog Number:** PM 317 DS AA

**Description:** 6.0 ml, prediluted

**Dilution:** Ready-to-use

**Diluent:** N/A

### Intended Use:

For In Vitro Diagnostic Use

GCDFP-15 + Mammaglobin is intended for laboratory use in the qualitative identification of GCDFP-15 and Mammaglobin proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

### Summary and Explanation:

Gross cystic disease fluid protein (mouse monoclonal) is a secretion from breast composed of several glycoproteins, including GCDFP-15. It is considered to be a marker of apocrine differentiation. Numerous studies have shown GCDFP-15 (BRST -2) to be a specific marker for breast cancer in formalin-fixed paraffin-embedded tissues and in cytologic preparation (fine needle aspirates). Other types of tissues that express GCDFP-15 are axillary sweat glands and submandibular salivary glands.

Mammaglobin (rabbit monoclonal), a mammary-specific member of the uteroglobin family, is known to be overexpressed in human breast cancer. Studies suggest that mammaglobin is one of the first relatively mammary-specific and mammary-sensitive markers. In normal breast tissue, mammaglobin labels breast ductal and lobular epithelial cells. However, mammaglobin is expressed in a higher percentage of lobular carcinoma versus ductal cell carcinoma. Studies have also shown that mammaglobin was not altered at the metastatic lymph node site. Mammaglobin has been shown to be expressed in non-breast cancer sites such as endometroid carcinomas (39%), endocervical adenocarcinoma in situ (45%), sweat gland carcinomas (40%), salivary gland carcinoma (20%), melanoma (6%) and is also found in a small percentage of ovarian carcinomas and pancreatic adenocarcinomas.

Mammaglobin is expressed in 50-60% of metastatic breast cancers while GCDFP-15 is expressed in approximately 20-25%. Mammaglobin is a more sensitive marker than GCDFP-15 for breast carcinoma; however, it lacks the specificity of GCDFP-15. The combination of GCDFP-15 and Mammaglobin and other markers may help to establish the correct interpretation of metastatic breast carcinoma.

### Principle of Procedure:

This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

**Source:** Mouse monoclonal and Rabbit monoclonal

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

**Clone:** D6 (GCDFP-15) and 31A5 (Mammaglobin)

**Isotype:** IgG2a (GCDFP-15) and IgG (Mammaglobin)

**Epitope/Antigen:** GCDFP-15 and Mammaglobin

### Cellular Localization:

GCDFP-15 (Cytoplasmic): Brown

Mammaglobin (Cytoplasmic): Red

**Positive Control:** Breast

### 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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

### Protocol Recommendations:

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment Solution (recommended):** Diva

### Pretreatment Protocol:

Heat Retrieval Method:

Preheat the retrieval solution to 95°C for 30 minutes in Biocare's Decloaking Chamber. Then, place slides into the preheated solution and retrieve under pressure at 95°C for 40 minutes; alternatively, steam tissue sections for 45-60 minutes or use a water bath at 95°C for 40 minutes. Allow solution to cool for 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 minutes at RT.

### Double Stain Detection:

Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.

**Chromogen (1):** Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

**Chromogen (2):** 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 Notes:

This antibody has been standardized with Biocare's MACH 2 Double Stain 2. It can also be used on an automated staining system. Use TBS buffer for washing steps. \* For optimum results, breast tissues should be fixed for 8-24 hours.

### Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

### Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

### 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) (4)
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 in contact with sensitive areas, wash with copious amounts of water. (5)
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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### References:

1. Bhargava R, Beriwal S, Dabbs DJ. Mammaglobin vs GCDFP-15: An Immunohistologic Validation Survey for Sensitivity and Specificity. *Am J Clin Pathol.* 2007 Jan; 127(1):1-11.
2. Wick MR, *et al.* Gross cystic disease fluid protein-15 as a marker for breast cancer: immunohistochemical analysis of 690 human neoplasms and comparison with alpha-lactalbumin. *Hum Pathol.* 1989 Mar; 20(3):281-7.
3. Han JH, *et al.* Mammaglobin expression in lymph nodes is an important marker of metastatic breast carcinoma. *Arch Pathol Lab Med.* 2003 Oct; 127(10):1330-4.
4. 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."
5. 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.

### Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.