



## CD44

### Prediluted Monoclonal Antibody

Control Number: 901-380-073013

ISO  
9001&13485  
CERTIFIED

**Catalog Number:** PM 380 AA

**Description:** 6.0 ml, prediluted

**Dilution:** Ready-to-use

**Diluent:** N/A

**Intended Use:**

For In Vitro Diagnostic Use

CD44 [BC8] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of CD44 protein 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:**

CD44 is a transmembranous glycoprotein (80 kDa) responsible for cell adhesion that is expressed in a wide range of normal tissues, such as tonsil, breast, prostate, skin, bladder and cervical squamous epithelium (1). Studies have shown that CD44, also known as HCAM, plays an important role in the cascade of metastasis and progression of human malignant tumors (2). In breast cancer studies, evaluation of CD44 expression has been useful in the differentiation of benign and malignant papillary lesions (2). Expression of CD44s, the most common CD44 isoform, has been reported to be associated with increased survival of patients with node-negative invasive breast carcinoma (3). Further studies have shown a subpopulation of CD44+/CD24- in breast cancer cells have stem/progenitor cell properties (4).

The CD44 predilute (PM380) has been titered specifically for use in bladder cancer. It may be used as an optional step with Uro-2™ (CK20 + p53) Multiplex Cocktail (API3001DS).

**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, an enzyme labeled polymer is then added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

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

**Clone:** BC8

**Isotype:** IgG2a

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** CD44

**Cellular Localization:** Cytoplasmic/cell membrane

**Positive Control:** Breast cancer or tonsil

**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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

**Protocol Recommendations:**

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

**Pretreatment Solution (recommended):** Diva

**Pretreatment Protocol:**

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water; alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

**Protocol Recommendations Cont'd:**

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

**Primary Antibody:** Incubate for 30 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 standardized with Biocare's MACH 2 detection system. It can also be used on an automated staining system and with other Biocare polymer detection kits. Use TBS buffer for washing steps.

**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) (5)
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. (6)
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 MSDS is available upon request and is located at <http://biocare.net/support/msds/>.

**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.



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1. Fox SB, *et al.* Normal human tissues, in addition to some tumors, express multiple different CD44 isoforms. *Cancer Res.* 1994 Aug 15;54(16):4539-46.
2. Tse GM, *et al.* CD44s is useful in the differentiation of benign and malignant papillary lesions of the breast. *J Clin Path.* 2005 Nov; 58(11):1185-8.
3. Diaz LK, *et al.* CD44 expression is associated with increased survival in node-negative invasive breast carcinoma. *Clin Cancer Res.* 2005 May 1;11 (9):3309-14.
4. Balic M, *et al.* Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. *Clin Cancer Res.* 2006 Oct 1;12 (19):5615-21.
5. 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."
6. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory workers from occupationally Acquired Infections; Approved guideline-Third Edition CLSI document M29-A3 Wayne, PA 2005.

