

# Ki-67 + CK7/8/18

Prediluted Multiplex Antibody Reagent  
901-3192DS-090817

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

**Catalog Number:** API 3192DS AA  
**Description:** 6.0 ml, prediluted  
**Dilution:** Ready-to-use  
**Diluent:** N/A

## Intended Use:

For In Vitro Diagnostic Use

Ki-67 + CK7/8/18 is a cocktail of mouse and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of Ki-67 and low molecular weight cytokeratin proteins (CK 7, 8, 18) 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:

The Ki-67 nuclear antigen is associated with cell proliferation and found throughout the cell cycle; though not in G0 phase (1,2). The assessment of Ki-67 proliferation in breast cancers has shown the Ki-67 labelling index is an important predictor of survival (3). Cytokeratin (CK) 7 is expressed in epithelial cells of the ovary, lung and breast. Rabbit monoclonal CK7 (Clone BC1) is often used in conjunction with CK20 and CDX-2 in distinguishing pulmonary, ovarian and breast carcinomas (CK7+) from most colon carcinomas (CK7-) (4). Clones EP17 and EP30 recognize CK8 and CK18, both of which recognize low molecular weight intermediate filament proteins. In normal tissues, CK8/18 recognizes all simple and glandular epithelium (5). In neoplastic tissues, CK8/18 may prove useful for the identification of most adenocarcinomas and some squamous cell carcinomas. CK8/18 expression patterns may also aid in the classification of tumors of unknown origin and poorly differentiated carcinomas (5-7). CK8/18 rabbit monoclonal may be useful as a staining mask in a multiplex stain with mouse monoclonal antibody Ki-67.

## 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.

## Reagent Provided:

Ki-67 + CK7/8/18 is provided as a prediluted antibody cocktail of anti-Ki-67, anti-CK7, anti-CK8 and anti-CK18 antibodies in buffer with carrier protein and preservative.

Antibody	anti-Ki-67	anti-CK7	anti-CK8	anti-CK18
Clone	MIB-1	BC1	EP17	EP30
Source	Mouse monoclonal	Rabbit monoclonal	Rabbit monoclonal	Rabbit monoclonal
Isotype	IgG/kappa	IgG	IgG	IgG
Epitope/Antigen	1002 bp Ki-67 cDNA fragment	CK7	CK8	CK18
Cellular Localization	Nuclear	Cytoplasmic	Cytoplasmic	Cytoplasmic
Staining	Brown (DAB)	Red (Warp Red)	Red (Warp Red)	Red (Warp Red)

## 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.

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

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

**Positive Tissue Control:** Breast carcinoma

## 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 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 20 minutes and then wash in distilled water.

**Protein Block:** Incubate for 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 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

**Chromogen (2):** Incubate for 5 minutes at RT with Biocare's DAB.

**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 Double Stain 2. 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.

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### Limitations Cont'd:

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](http://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) (8)
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. (9)
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>.

### 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.

### References:

1. Gerdes J, *et al.* Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol.* 1984 Oct;133(4):1710-5.
2. McCormick D, *et al.* Detection of the Ki-67 antigen in fixed and wax-embedded sections with the monoclonal antibody MIB1. *Histopathology.* 1993 Apr;22(4):355-60.
3. Pinder SE, *et al.* Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. *Br J Cancer.* 1995 Jan;71(1):146-9.
4. Qi W, Tacha D. Characterization and applications of a newly developed rabbit monoclonal antibody to cytokeratin 7 (CK7) for immunohistochemistry. *Appl Immunohistochem Mol Morphol.* 2009 May;17(3):233-8.

5. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. *Histochem Cell Biol.* 2008 Jun;129(6):705-33.
6. Dabbs DJ. *Diagnostic Immunohistochemistry.* Philadelphia,PA: Churchill Livingstone, 2002.
7. Makino T, *et al.* Cytokeratins 18 and 8 are poor prognostic markers in patients with squamous cell carcinoma of the oesophagus. *Br J Cancer.* 2009 Oct 20;101(8):1298-306.
8. 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."
9. 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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