

**Estrogen Receptor (ER) [6F11 + SP1]**  
Prediluted Mouse Monoclonal and Rabbit Monoclonal Antibody  
Control Number: 901-308-061114

ISO  
9001&13485  
CERTIFIED

**Catalog Number:** API 308 AA, H  
**Description:** 6.0, 25 ml, prediluted  
**Dilution:** Ready-to-use  
**Diluent:** N/A

**Intended Use:**

For In Vitro Diagnostic Use  
Estrogen Receptor (ER) [6F11 + SP1] is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that are intended for laboratory use in the qualitative identification of estrogen receptor (ER) 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.

**FOR DISTRIBUTION OUTSIDE THE UNITED STATES ONLY.**

**Summary and Explanation:**

Estrogen Receptor (ER) [6F11 + SP1] is a cocktail of mouse monoclonal antibody [6F11] and rabbit monoclonal antibody [SP1] directed against human estrogen receptor (ER) protein. ER is a 66 kDa protein that mediates the actions of estrogen in estrogen-responsive tissues. The ER gene consists of more than 140 kb of genomic DNA divided into 8 exons. These translate into a protein with six functionally discrete domains, labeled A through F. Both antibodies have been used for immunohistochemistry on formalin-fixed paraffin-embedded tissues (1-7).

**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 secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal and Rabbit monoclonal

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

**Clone:** 6F11 + SP1

**Isotype:** IgG1/kappa (6F11) and IgG (SP1)

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

**Epitope/Antigen:** Estrogen receptor protein

**Cellular Localization:** Nuclear

**Positive Tissue Control:** Breast carcinoma

**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):** Reveal or 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 45-60 minutes at RT.

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

**Protocol Recommendations Cont'd:**

**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 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 4 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. For optimum results breast tissues should be fixed a minimum of 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) (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.

Biocare Medical, LLC.

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1. Bevitt DJ, *et al.* New monoclonal antibodies to oestrogen and progesterone receptors effective for paraffin section immunohistochemistry. *J Pathol.* 1997 Oct;183(2):228-32.
2. Kaplan PA, *et al.* 1D5 and 6F11: An immunohistochemical comparison of two monoclonal antibodies for the evaluation of estrogen receptor status in primary breast carcinoma. *Am J Clin Pathol.* 2005 Feb;123(2):276-80.
3. Bogina G, *et al.* Comparison of anti-estrogen receptor antibodies SP1, 6F11, and 1D5 in breast cancer: lower 1D5 sensitivity but questionable clinical implications. *Am J Clin Pathol.* 2012 Nov;138(5):697-702.
4. Cheang MC, *et al.* Immunohistochemical detection using the new rabbit monoclonal antibody SP1 of estrogen receptor in breast cancer is superior to mouse monoclonal antibody 1D5 in predicting survival. *J Clin Oncol.* 2006 Dec;24(36):5637-44.
5. Rosso S, *et al.* Rabbit monoclonal antibodies: a comparative study between a novel category of immunoreagents and the corresponding mouse monoclonal antibodies. *Am J Clin Pathol.* 2005 Aug; 124(2):295-302.
6. Cano G, *et al.* Estimation of hormone receptor status in fine-needle aspirates and paraffin-embedded sections from breast cancer using the novel rabbit monoclonal antibodies SP1 and SP2. *Diagn Cytopathol.* 2003 Oct;29(4):207-11.
7. Rocha R, *et al.* Rabbit monoclonal antibodies show higher sensitivity than mouse monoclonals for estrogen and progesterone receptor evaluation in breast cancer by immunohistochemistry. *Pathol Res Pract.* 2008;204(9):655-62.
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-Third Edition CLSI document M29-A3 Wayne, PA 2005.

