

Estrogen Receptor (ER) [SP1]
Concentrated and Prediluted Rabbit Monoclonal Antibody
Control Number: 901-301-081817

Catalog Number:	ACI 301 A, B, C	API 301 AA	OAI 301 T60
Description:	0.1, 0.5, 1.0 ml, concentrated	6.0 ml, prediluted	60 tests, prediluted
Dilution:	1:50-1:100	Ready-to-use	Ready-to-use
Diluent:	Renoir Red	N/A	N/A

Intended Use:

For In Vitro Diagnostic Use
Estrogen Receptor (ER) [SP1] is a rabbit monoclonal antibody that is 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.

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Summary and Explanation:

Human estrogen receptor (ER) is a 66 kDa protein that acts as an estrogen-dependent, nuclear hormone receptor. Studies have shown ER is present in the nuclei of epithelial cells in normal breast and endometrial tissues, as well as a subset of breast carcinomas. The SP1 clone is a high affinity rabbit monoclonal antibody directed against an epitope of the C-terminus of the ER protein (1). SP1 has been shown to stain formalin-fixed paraffin-embedded tissues. The robustness of SP1 has been demonstrated by successful immunohistochemistry using lower temperatures for antigen retrieval (e.g. 80 or 95°C), and in some instances, staining can be obtained even without antigen retrieval (2-3).

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. This detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Rabbit monoclonal

Species Reactivity: Human; others not tested

Clone: SP1

Isotype: IgG

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

Protocol Recommendations (manual use):

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.

Probe: N/A

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

Protocol Recommendations (manual use) Cont'd:

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.

Protocol Recommendations (ONCORE Automated Slide Staining System):

OAI301 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: ER Rb

Protocol Template (Description): Rb HRP Template 1

Dewaxing (DS Option): DS2

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

Reagent Name, Time, Temp.: ER Rb, 30 min., 25°C

Technical Note:

This antibody has been optimized for use with Biocare's MACH 2 Rabbit HRP-Polymer Detection and ONCORE HRP Detection. Other Biocare polymer detection kits may be used; however, users must validate incubation times and protocols for their specific application. 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 (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) (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 into contact with sensitive areas, wash with copious amounts of water. (6)

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Precautions Cont'd:

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/>.

References:

1. 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.
2. Rossi 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.
3. 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.
4. 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.
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-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.