VENTANA®

CONFIRM™ anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody

Catalog number 790-4324 (50 tests)
790-4325 (250 tests)

INDICATIONS AND USE

Intended Use

This antibody is intended for *in vitro* diagnostic (IVD) use.
Ventana Medical Systems' (Ventana) CONFIRM™ anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody is a rabbit monoclonal antibody (IgG) that is intended for laboratory use for the qualitative detection of estrogen receptor (ER) antigen in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide stainer with Ventana detection kits and ancillary reagents. This antibody has been optimally diluted for use with Ventana *i*VIEW™ DAB detection kit. Light microscopy is used to detect the staining of cell components.
CONFIRM anti-ER (SP1) is directed against an epitope present on human ER alpha protein located in the nucleus of ER positive normal and neoplastic cells. CONFIRM anti-ER (SP1) is indicated as an aid in the management, prognosis, and prediction of therapy outcome of breast cancer.
The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. Prescription only.

Summary and Explanation

CONFIRM anti-ER (SP1) is a rabbit monoclonal antibody that recognizes human estrogen receptor alpha. A synthetic peptide corresponding to the C-terminal portion of the ER molecule was used as the immunogen.[1] CONFIRM anti-ER (SP1) has been shown to react with 66 kD protein from MCF-7 cells via Western blotting.[1] The protein size is in agreement with that predicted from the cloning of the gene for ER.[2]

Determination of ER status for all primary breast carcinomas was recommended by the NIH in 1979, in order to better determine appropriate therapy. In 1985, both the NIH and the American Cancer Society independently published reports in support of determining hormone receptor status as an aid in the management of breast cancer. A number of methodologies to asses ER status have been in use. FDA cleared therapies include cytosol receptor assay (SBA/DCC) analyzed by Scatchard plot (1981), histochemical analysis of tissue using fluorescent microscopy, histochemical analysis of frozen tissue using anti-ER rat monoclonal antibody conjugate (1988), and enzyme immunoassay (EIA) also using anti-ER rat monoclonal antibody conjugate (1988).[3] The immunohistochemical detection of ER has been described in cultured human breast cells,[4] some human breast cancer tissues,[4,5] human endometrium,[6] some endometrial cancers,[7] some low grade endometrial stromal sarcomas,[8] some cultured endometrial cells,[9] some sweat gland tumors,[10] some benign thyroid disease tissues,[11] some thyroid cancers,[12] some gastric cancers,[13,14] some prostatic carcinomas[15] and some female human bladders.[16]
Staining results with CONFIRM anti-ER (SP1) in normal tissues, neoplastic tissues, and 198 cases of breast carcinoma were evaluated by Ventana. In the 87 normal tissues tested, expression was consistent with the published literature in that ER was localized to the nucleus, and expression was limited to reproductive tissues (breast, cervix, endometrium, prostate, and uterus).[17]
Detection of ER in 198 cases of breast carcinoma was evaluated in a comparative study using CONFIRM anti-ER (SP1) and CONFIRM anti-ER (6F11) with *i*VIEW DAB Detection Kit. The estimated overall agreement between the two antibodies was 92%. The Kappa test for agreement returned a value of 0.81,indicating good agreement. See Summary of Expected Results for details and additional performance information.

Breast cancer is the most common carcinoma occurring in women, and the second leading cause of cancer related death. In North America, a woman's chance of developing breast cancer is one in eight.[18] Early detection and appropriate treatment therapies can significantly affect overall survival.[19,20] Small tissue samples may be easily used in routine immunohistochemistry (IHC), making this technique, in combination with antibodies that detect antigens important for carcinoma interpretation, an effective tool for the pathologist in diagnosis and prognosis of disease. An important marker in breast cancer today is estrogen receptor (ER), which binds estrogen with high affinity and specificity. ER is found in target tissue cells, including the breast where they act as stimulators of various biological processes when bound by estrogen. Lowering of blood estrogen levels in turn reduces biological activity of target cells. This has formed the basis of endocrine therapy for women with breast carcinoma that are positive for ER. Various surgical approaches to lowering estrogen levels may also be used, including ovariectomy, hypophysectomy, and adrenalectomy.[8]

A high ER concentration on the mammary tumor correlates with greater response to endocrine therapy.[12] Conversely, the absence of ERs would render such therapy inappropriate. Thus the knowledge of ER status plays an important role in the selection of treatment for the patient (but is not the sole basis for treatment selection).[19] Currently, the treatment of choice for ER positive carcinomas is tamoxifen.[19,21] Knowledge of ER status in breast tumors also aids in prognosis and treatment of the patient.[20] It has been shown in a number of studies that the presence of ER confers a favorable long term prognosis.[19,22, 23, 24] If remission occurs, ER status must be reassessed, as it can change over time.[25]
It has also been suggested that an assay for ER, in conjunction with tests for other biological markers, may prove useful in determining the origin of metastatic breast cancer, particularly when detected in the lung and gastrointestinal tract.[25] Other investigators, however, have found that lymph node metastases did not always maintain ER positivity.[25] Interpretation of the results of any detection system for ER must take into consideration the heterogeneity of breast cancer tumors. Tumors frequently contain benign epithelial cells from normal hyperplasic lobules or ducts that are also positive for ER. This tests utilizing tissues homogenates such as DCC or EIA may not be solely a reflection of ER status in malignant tissue.[27] Histological tissue preparations have the advantage of intact tissue morphology to aid in the interpretation of the ER positivity of the sample. All histological tests should be interpreted by a specialist in breast cancer morphology, pathology or both, and the results should be used in conjunction with other clinical and laboratory data.

Principles and Procedures

CONFIRM anti-ER (SP1) binds to ER in paraffin embedded tissue sections. The specific antibody can be localized by either a biotin conjugated secondary antibody formulation that recognizes rabbit immunoglobulins, followed by the addition of a streptavidin horseradish peroxidase (HRP) conjugate (*i*VIEW DAB Detection Kit) or a secondary antibody-HRP conjugate (*ultra*View™ DAB Detection Kit). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the Ventana automated slide stainer washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies Liquid Coverslip, which minimizes evaporation of the aqueous reagents from the specimen slide.
Clinical cases should be evaluated within the context of the performance of appropriate controls. Ventana recommends the inclusion of a positive tissue control fixed and processed in the same manner as the patient specimen (for example, a weakly positive breast carcinoma or uterus). In addition to staining with CONFIRM anti-ER (SP1), a second slide should be stained with Ventana CONFIRM Negative Control Rabbit Ig. For the test to be considered valid, the positive control tissue should exhibit nuclear staining of the tumor cells or uterine glands and stroma. These components should be negative when stained with CONFIRM Negative Control Rabbit Ig. In addition, it is recommended that a negative tissue control slide (for example, an ER negative breast carcinoma) be included for every batch of samples processed and run on the Ventana automated slide stainer. This negative tissue control should be stained with CONFIRM anti-ER (SP1) to ensure that the antigen enhancement and other pretreatment procedures did not create false positive staining.

MATERIALS AND METHODS

Reagents Provided

Catalog #790-4324: CONFIRM anti-ER (SP1) contains sufficient reagent for 50 tests.
1 – 5 ml dispenser of CONFIRM anti-ER (SP1) contains approximately 5 µg of a rabbit monoclonal antibody directed against human ER antigen.

Catalog #790-4325: CONFIRM anti-ER (SP1) contains sufficient reagent for 250 tests.
1 – 25 ml dispenser CONFIRM anti-ER (SP1) contains approximately 25 µg of a rabbit monoclonal antibody directed against human ER antigen.

The antibody is diluted in 0.05 M Tris-HCl with 2% carrier protein, and 0.10% ProClin 300, a preservative containing the active ingredients 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one. There is trace (~0.2%) fetal calf serum of U.S. origin from the stock solution.
Total protein concentration of the reagent is approximately 20 mg/ml. Specific antibody concentration is approximately 1 µg/ml. CONFIRM anti-ER (SP1) is a rabbit IgG. There is no known irrelevant antibody in this product.

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on a Ventana automated slide stainer in combination with Ventana *i*VIEW DAB Detection Kit, and is compatible with *ultra*View DAB Detection Kit. No reconstitution, mixing, dilution, or titration is required.
Further dilution may result in loss of antigen staining. The user must validate any such changes. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and require regular use of controls.

Materials and Reagents Needed But Not Provided

The following reagents and materials may be required for staining but are not provided:

1. Ventana CONFIRM Negative Control Rabbit Ig
2. Microscope slides, positively charged
3. Positive and negative tissue controls
4. Drying oven capable of maintaining a temperature of 70° C ± 5° C
5. Bar code labels (appropriate for negative control and primary antibody being tested)

6. 10% neutral buffered formalin
7. Staining jars or baths
8. Tissue-Tek® staining dishes
9. Timer
10. Xylene
11. Ethanol or reagent alcohol
12. Deionized or distilled water
13. Biocare Medical's Decloaking Chamber (NexES® IHC automated slide stainers)
14. NexES IHC, BenchMark® Series automated slide stainers
15. Ventana *i*VIEW DAB or *ultra*View DAB detection kits
16. Ventana Endogenous Biotin Blocking Kit*
17. Ventana APK Wash (10X)* (NexES IHC automated slide stainers)
18. Ventana Liquid Coverslip™ (Low Temperature) (NexES IHC automated slide stainers)
19. Ventana EZ Prep™ (10X)* (BenchMark Series automated slide stainers)
20. Ventana Reaction Buffer (10X)* (BenchMark Series automated slide stainers)
21. Ventana Liquid Coverslip (High Temperature) (BenchMark Series automated slide stainers)
22. Ventana Cell Conditioning 1 (Pre-dilute) (BenchMark Series automated slide stainers)
23. Ventana Hematoxylin II counterstain
24. Ventana Bluing Reagent
25. Mounting medium
26. Cover glass
27. Light microscope (20-60X)

* As needed for specific applications.

Storage and Handling

Store at 2-8° C. Do not freeze. The user must validate any storage conditions other than those specified in the package insert.

CONFIRM anti-ER (SP1) should be allowed to stand at least 30 minutes at room temperature prior to use. To ensure proper reagent delivery and stability of the antibody after every run, the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date for the prescribed storage method. The product has been designed to have 12 months dating after the date of manufacture.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Your local Ventana office should be contacted immediately if there is an indication of reagent instability.

Specimen Collection and Preparation for Analysis

Formalin fixed, paraffin embedded tissues which have been antigen enhanced are suitable for use with CONFIRM anti-ER (SP1) when used with Ventana detection kits and a Ventana automated slide stainer (see Materials and Reagents Needed But Not Provided).

The recommended fixative is 10% neutral buffered formalin. The amount used is 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24 hour period. A 3 mm or smaller section of tissue should be fixed no less than 4 hours and no more than 8 hours. Fixation can be performed at room temperature (15-25 C).[28]

Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades.[28]

Approximately 4-5 µm *thick* sections should be cut and picked up on glass slides. The slides should either be silanized or coated with a polylysine compound. Tissue should be dried by placing the slides in a 70°C (±5°C) oven for at least 2 hours, but not longer than 24 hours.[28]

Manual Deparaffinization Procedure

Required when using the NexES IHC automated slide stainer or if deparaffinization is not selected on a BenchMark Series automated slide stainer:

1. For instructions on when to label slides with barcode label, refer to the Instructions for Use section of the specific automated slide stainer Operator's Manual.
2. Immerse the slides sequentially in 3 xylene baths for 5±1 minutes each.
3. Transfer the slides to 100% ethanol and immerse sequentially in 2 baths for 3±1 minutes each.
4. Transfer the slides to 95% ethanol and immerse them in a bath of this solution for 3±1 minutes.
5. Transfer the slides to 80% ethanol and immerse them in this solution for 3±1 minutes.
6. Transfer the slides to a bath of deionized or distilled water and dip a minimum of 10 times.
7. Transfer slides to APK Wash (1X) or buffer solution as appropriate. For APK Wash, the slides should remain until you are ready to perform the staining run. For buffer solution, the slides should remain until you are ready to perform the antigen unmasking procedure. Do not allow the slides to dry.

Slides stained on the BenchMark Series automated slide stainers can be deparaffinized on the instrument. If this option is selected, apply barcode labels to slides and place slides on the instrument. If the option is not selected, follow the Manual Deparaffinization Procedure above.

WARNINGS AND PRECAUTIONS

1. Take reasonable precautions when handling reagents. Use disposable gloves when handling suspected carcinogens or toxic materials (example: xylene or formaldehyde).
2. Do not smoke, eat or drink in areas where specimens or reagents are being handled.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
5. Avoid microbial contamination of reagents, as this could produce incorrect results.
6. Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change.
7. The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such change.
8. The preservative in the reagent is ProClin 300. Symptoms of overexposure to ProClin 300 include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin 300 in this product is 0.10% and does not meet the OSHA criteria for a hazardous substance. Systemic allergic reactions are possible in sensitive individuals.
9. Consult local or state authorities with regard to recommended method of disposal.

INSTRUCTIONS FOR USE

Step by Step Procedure

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. Recommended staining protocols for the automated slide stainers are listed below in Table 1. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the Operator's Manual. Other operating parameters for the automated slide stainers have been preset at the factory.

Table 1. Recommended Staining Protocols for CONFIRM anti-ER (SP1)

Procedure Type	Platform/Method	
	NexES IHC	BenchMark Series
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	1 mM EDTA (pH 8.0), 2 minutes, Decloaking Chamber 120°C	Cell Conditioning 1, Standard
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 8 to 32 minutes, 37°C	Approximately 8 to 32 minutes, 37°C
A/B Block (Biotin Blocking)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin II, 4 minutes	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes	Bluing, 4 minutes

The procedures for staining on the Ventana automated slide stainers are as follows. For more detailed instructions and additional protocol options, refer to your Operator's Manual.

NexES IHC Automated Slide Stainers

Antigen Unmasking Required:

1. Slides are to be deparaffinized through a series of xylene and gradient alcohols to water and then to appropriate buffer. Perform antigen unmasking procedure and transfer slides to APK Wash (1X).
2. Load the primary antibody, appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place the reagent tray on the automated slide stainer. Check bulk fluids and waste.
3. Dry the painted end of the slide and then apply slide barcode label that corresponds to the antibody protocol to be performed.
4. Load the deparaffinized, antigen unmasked, labeled slides from the APK Wash (1X). Avoid tissue drying.

Manual Antigen Unmasking Procedure

Manual antigen unmasking is required when using the NexES IHC automated slide stainer. Antigen enhancement (cell conditioning) procedure (for tissue slides to be stained on NexES IHC:

1. Prepare the Decloaking Chamber for use.
2. Place the pan into the chamber. Note: Make sure that the outside of the pan is completely dry prior to placing it in the chamber. If the outside of the pan is wet, the Decloaking Chamber will make a cracking noise and any water in the chamber will cause a malfunction.

3. Align the handles of the pot with the handles of the chamber.
4. Fill the pan with 500 ml of deionized water and place the heat shield, (circular screen), in the center of the pot. Note: The heat shield keeps the plastic containers from warping.
5. Place each Tissue-Tek staining dish, filled with 250 ml of 1 mM EDTA (pH 8.0) and the appropriate slides on the heat shield which is placed in the center of the pan. Up to 2 containers may be placed in the chamber, but make sure both are touching the heat shield.
6. Put the lid on the Decloaking Chamber and secure (align the open arrow with the white dot on the pan handle. Grip the lid handle, and rotate clockwise to the closed position; when the lid is locked in the proper position, the Vent Lever will lower the weight on the vent nozzle).
7. Turn the rheostat to 10 and lock into place (approximately 120°C).
8. Turn on the Decloaking Chamber and monitor until the pressure reaches 17-25 psi and the temperature is 120°-125°C. Once the Decloaking Chamber reaches the desired temperature, time for 2 minutes using a calibrated manual timer, as the Decloaking Chamber timer is not "real time" consistent. When the manual timer goes off, turn the Decloaker timer to the off position. The heat will turn off and the light will turn from "heat on" to "keep warm". Note: Technician must monitor temperature and pressure conditions to confirm desired specifications are met.
9. Once the antigen enhancement procedure is completed, turn off the Decloaking Chamber.
10. The technician can monitor the declining pressure by periodically checking the pressure gauge. When pressure reaches 0 psi, the Decloaking Chamber can be opened safely. Rotate the lid counterclockwise and remove it slowly, allowing steam to escape away from your hand. Note: Be very careful when opening lid, as surface and liquid temperatures remain high.
11. Remove the container of slides from the pan and place slide holders containing processed slides in a container of room temperature, deionized water.
12. Once rinsing is complete, place the slides in a Tissue-Tek slide rack filled with deionized water for maintaining hydration while barcode labels are applied to slides. One by one, remove the slides from the slide rack and blot the frosted end dry, ensuring the tissue sections do not dry during the process. Label each slide with the appropriate barcode label, and return it to the slide container. Repeat this process for all slides.
13. Once all slides have been labeled, empty the deionized water from the slide container and refill it with APK Wash (1X). Slides should remain in this solution until ready to perform staining run. NOTE: Slides must be stained within 4 hours of being cell conditioned. They may be left in wash solution for up to 2 hours if necessary, as long as tissue is not allowed to dry. Blot dry frosted end of processed tissue slides, ensuring that the tissue sections do not dry. Properly label processed slides with barcodes and place in APK Wash until ready to load on Ventana NexES automated slide stainer.

BenchMark Series Automated Slide Stainers

1. Apply slide barcode label that corresponds to the antibody protocol to be performed.
2. Load the primary antibody, appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place the reagent tray on the automated slide stainer. Check bulk fluids and waste.
3. Load the slides onto the automated slide stainer.

For All Instruments

1. Start the staining run.
2. At the completion of the run, remove the slides from the automated slide stainer.
3. Wash in a mild dishwashing detergent or alcohol to remove the coverslip solution; dehydrate, clear, and coverslip with permanent mounting media in the usual manner.

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. An example of tissue to use as a positive control with CONFIRM anti-ER (SP1) is a weakly positive breast carcinoma. The positive staining cells or tissue components (nuclear staining of tumor cells) are used to confirm that CONFIRM anti-ER (SP1) was applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Such tissues may monitor all steps of the procedure, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable than strong positive staining for optimal quality control and for detecting minor levels of reagent degradation. Ideally, a breast carcinoma tissue which is known to have weak, but positive staining should be chosen to ensure that the system is sensitive to small amounts of reagent degradation or problems with the IHC methodology.

Alternatively, normal human proliferative endometrium may be used for a positive control. The positive staining components are nuclear staining of the glandular epithelia, and stromal and smooth muscle cells. Endometrial tissue, however, may not stain weakly enough to detect small amounts of reagent degradation or problems with the IHC methodology.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, and not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control

Use a tissue control known to be fixed, processed and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of CONFIRM anti-ER (SP1) for demonstration of ER, and to provide an indication of specific background staining (false positive staining). Also the variety of different cell types in most tissue sections can be used by the laboratorian as internal negative control to verify CONFIRM anti-ER (SP1) performance specifications. For example, the same tissue (endometrium) used for the positive tissue control may be used as the negative tissue control. The components that do not stain (cytoplasm, cell membrane) should show absence of specific staining in cells not expected to stain, and provide an indication of specific background staining. Alternatively, normal human tonsil may be used. There should be no positive staining (lymphoid cells, mucosal cells and connective tissue). The negative tissue control also should be used as an aid in interpretation of results. The variety of different cell types present in most tissue sections frequently offers negative control sites, but this should be verified by the user. If specific staining occurs in the negative tissue control sites, results with the patient specimens should be considered invalid.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local Ventana office immediately. If quality control results do not meet specifications, patient results are invalid. If discrepancies occur, refer to the Troubleshooting section of this insert. Identify and correct the problem, then repeat the patient samples.

Negative Reagent Control

A negative reagent control must be run for every specimen to aid in the interpretation of results. A negative reagent control is used in place of the primary antibody to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. This provides an indication of nonspecific background staining for each slide. In place of the primary antibody, stain the slide with CONFIRM Negative Control Rabbit Ig, a purified non-immune rabbit IgG not reacting with human specimens. If an alternative negative reagent control is used, dilute to the same dilution as the primary antibody antiserum with Ventana Antibody Diluent. Approximately 0.2% fetal calf serum is retained in the CONFIRM anti-ER (SP1). Addition of 0.2% fetal calf serum in Ventana Antibody Diluent is also suitable for use as a nonspecific negative reagent control. The incubation period for the negative reagent control should equal the primary antibody.

When panels of several antibodies are used on serial sections, a negative reagent control on one slide may serve as a negative or nonspecific binding background control for other antibodies.

Assay Verification

Prior to initial use of this antibody in a diagnostic procedure, or if there is a change of lot number, the specificity of the antibody should be verified by staining a number of positive and negative tissues with known performance characteristics. Refer to the quality control procedures previously outlined in this section of the product insert and to the quality control recommendations of the CAP certification program for immunohistochemistry as well as the NCCLS IHC guideline.[29,30] These quality control procedures should be repeated for each new antibody lot or whenever there is a change of lot number of one of the reagents in a matched set or a change in assay parameters. Quality control cannot be meaningfully performed on an individual reagent in isolation since the matched reagents, along with a defined assay protocol, must be tested in unison before using a kit for diagnostic purposes. Tissues listed in the Summary of Expected Results are suitable for assay verification. Assay verification on a daily basis may be accomplished through the proper use of the above mentioned positive and negative controls, as described in this section. In addition, it is recommended that on a monthly basis, the ER positive tissue control be stained and compared to the same tissue control stained the previous month. Comparison of controls stained at monthly intervals serves to monitor the assay stability, sensitivity, specificity, and reproducibility. All quality control requirements should be performed in conformance with local, state and federal regulations or accreditation requirements.

Interpretation of Results

The Ventana automated immunostaining procedure causes a colored reaction product to precipitate at the antigen sites localized by CONFIRM anti-ER (SP1). A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative controls and qualify the stained product before interpreting results.

Positive Tissue Control

The positive tissue control stained with CONFIRM anti-ER (SP1) should be examined first to ascertain that all reagents are functioning properly. The presence of a brown (3,3' diaminobenzidine tetrachloride, DAB) reaction product within the target cells' nuclei is indicative of positive reactivity. An example of a tissue that may be used as a positive control is a known weakly positive breast carcinoma, e.g. 11-25%. Nuclei of the tumor cells

should be positive, with the stroma remaining ER negative. It is imperative that only nuclear staining be considered positive if a false positive interpretation is to be avoided. Normal human endometrium may also be used. In normal endometrium, ER staining is seen in nuclei of the endometrial glands and stroma. If the positive tissue controls fail to demonstrate appropriate positive staining, any results with the test specimens should be considered invalid.

Negative Tissue Control

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. The breast carcinoma used as a positive control may also be used as a negative control tissue. Stromal elements should show no nuclear staining. Alternatively, normal human tonsil may be used. There should be no staining in the lymphoid cells, mucosal cells, or connective tissue. If specific staining occurs in the negative tissue control, results with the patient specimen should be considered invalid. Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in tissue sections that are excessively formalin fixed. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells will often stain nonspecifically.[31]

Patient Tissue

Patient specimens stained with CONFIRM anti-ER (SP1) should be examined last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. ER may be detected among other neoplasms, such as cancers of the ovary and endometrium.[8] Photomicrographs of appropriate ER staining in paraffin embedded breast carcinomas may be found in the scientific literature.[4] The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist. Refer to Summary and Explanation, Limitations, and Summary of Expected Results for specific information regarding immunoreactivity.

LIMITATIONS

General Limitations

1. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents and tissues, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be a consequence of variations in fixation and embedding methods, or from inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
5. Ventana provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. This product is not intended for use in flow cytometry, performance characteristics have not been determined.
7. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.[32] Contact your local Ventana office with documented unexpected reactions.
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[33]
9. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results due to autoantibodies or natural antibodies.
10. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.[34]
11. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

Specific Limitations

1. The antibody, in combination with Ventana detection kits and accessories, detects antigen that survives routine formalin fixation, tissue processing and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.
2. Primary antibody incubation time depends on the degree of tissue fixation and may range from 8 to 32 minutes. Ventana recommends 16 minutes for use with iVIEW DAB. For further information on fixation variables refer to Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist.[34]
3. A CONFIRM anti-ER (SP1) negative result does not exclude the presence of ER. Negative reactions in breast carcinomas may be due to loss or marked decrease of expression of antigen. Therefore, it is recommended that this antibody be used in a panel of antibodies including progesterone receptor.

SUMMARY OF EXPECTED RESULTS

Immunoreactivity

Immunoreactivity of CONFIRM anti-ER (SP1) was demonstrated by a study using clinical specimens that showed appropriate staining in formalin fixed, paraffin embedded breast carcinoma tissue stained using the recommended protocol. The results of 198 cases were compared to those of ER detected by IHC using Ventana antibody CONFIRM anti-ER (6F11).

External Design Validation Study: The cases were selected from an archived collection of breast carcinoma biopsy tissues fixed in 10% NBF and embedded in paraffin blocks. Samples were stained with CONFIRM anti-ER (SP1) on a BenchMark XT using the Ventana recommended conditions (see Table 2) and compared to a matched cohort of slides previously stained with CONFIRM anti-ER (6F11). Endogenous biotin was blocked using Ventana Endogenous Biotin Blocking Kit. The antibody was detected using iVIEW DAB Detection Kit.

Table 2. Run Conditions.

Procedure Type	Platform/Method
	BenchMark XT
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Biotin Blocking	Selected
Antibody (Primary)	ER (6F11) 32 min; ER (SP1) 16 minutes
Counterstain (Hematoxylin)	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

iVIEW DAB Detection Kit includes:

1. An inhibitor solution; added to reduce the endogenous peroxidase activity in the tissue.
2. Universal biotinylated secondary antibodies.
3. Streptavidin-HRPO.
4. DAB solution and hydrogen peroxide. The two reagents are applied together and mixed on the specimen slide.
5. Copper enhancement.

Quality control procedures were provided by the study laboratory and included a positive tissue control slide placed on every run. Each case had a single slide stained with CONFIRM anti-ER (SP1) and one slide with CONFIRM Negative Control Rabbit Ig. For the test to be considered valid, the positive control tissue was expected to exhibit nuclear staining. These components were to be negative when stained with CONFIRM Negative Control Rabbit Ig. A matched cohort of cases were stained with Anti-ER (6F11) as part of a previous study. The study cases were evaluated within the context of the performance of the controls.

Scoring Criteria: Staining of CONFIRM anti-ER (6F11) and CONFIRM anti-ER (SP1) was assessed as either positive or negative according to the clinical procedure at the study site. Cases were considered positive if 5% or more of the tumor cells were stained. Results were as follows:

Table 3: CONFIRM anti-ER (SP1) Compared to CONFIRM anti-ER (6F11)

		CONFIRM anti-ER (6F11)		
CONFIRM anti-ER (SP1)		Positive	Negative	Total
	Positive	132	12	144
	Negative	4	50	54
TOTAL		136	62	198

Discrepancy Resolution: Samples employed in this study were previously evaluated for progesterone receptor expression with CONFIRM anti-PR (1A6). Expression of ER in breast cancer specimen correlates strongly with co-expression of progesterone receptor as

progesterone is an estrogen inducible and dependent protein. The expression of PR is indicative of an intact estrogen receptor pathyway. Discrepant results obtained in the study between CONFIRM anti-ER (SP1) and CONFIRM anti-ER (6F11) were evaluated by reference to the progesterone receptor status as determined immunohistochemically with CONFIRM anti-PR (1A6). There were 4 cases that were CONFIRM anti-ER (SP1) negative and CONFIRM anti-ER (6F11) positive. Two of the 4 cases were positive for progesterone receptor expression and two were negative. There were 12 cases that were CONFIRM anti-ER (SP1) positive and CONFIRM anti-ER (6F11) negative. Seven of the 12 cases were positive for progesterone receptor expression and 5 of the 12 cases were negative. The positive agreement between CONFIRM anti-ER (SP1) and CONFIRM anti-ER (6F11) was 90%. The negative agreement between CONFIRM anti-ER (SP1) and CONFIRM anti-ER (6F11) was 81%. The estimated overall agreement was 92%. Applying the McNemar's test, the difference in proportion is 0.040. The 95%CI is -0.004 to 0.076 (exact) with the 2-tailed p value equal to 1.0000 (exact). The kappa statistic for agreement of CONFIRM anti-ER (SP1) and CONFIRM anti-ER (6F11) is 0.81.

Specificity: Immunoreactivity of CONFIRM anti-ER (SP1) was determined by a study that showed appropriate staining of ER antigen. The 87 normal tissues examined included: cerebrum, adrenal, ovary, pancreas, parathyroid, hypophysis, testis, thyroid, breast, spleen, tonsil, thymus, bone marrow, lung, heart, esophagus, stomach, intestine, colon, liver, salivary gland, kidney, prostate, cervix/uterine, skin, nerve, mesothelium, endometrium, skeletal muscle. Staining was nuclear, with one case of ovary showing unexpected negative staining. Positive nuclear staining included the lobular and ductal cells of the breast, the glandular epithelium and fibromuscular cells of the cervix/uterine, the glandular epithelium, stromal tissues, and smooth muscle cells of the endometrium, and the stromal cells of the prostate.

Ventana also tested a total of 51 formalin fixed, paraffin embedded neoplastic tissues with CONFIRM anti-ER (SP1), using the same protocols and pretreatment procedures as those used for the normal tissue testing. The tissues examined included neoplastic tissue from the following tissues: brain, ovary, pancreas, testis, thyroid, breast, spleen, lung, esophagus, stomach, instestine, colon, rectum, liver, kidney, prostate, uterine, uterine cervix, striated muscle, skin, mediastinum, retroperitoneum, abdominal cavity, bladder, cervical cancer, lymphoma. 1 out of 2 prostate cases, 1 out of 3 uterine cases, and 1 out of 2 uterine cervix cases were positive for ER.

Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity weakens ER detection by CONFIRM anti-ER (SP1) and may generate false negative results.

Intra run reproducibility of staining was determined by staining 10 slides containing the same tissue. Ten of 10 slides stained positively. All slides stained with similar percent positivity. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium, and high antigen density in a single run.

Inter run reproducibility of staining was determined by staining slides containing the same tissue on 9 different runs. Nine of 9 slides stained positively. All slides stained with similar percent positivity. Users should verify between run reproducibility results by staining several sets of serial sections with low, medium, and high antigen density on different days.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper barcode label. If the slide is labeled properly, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step should be included.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If specific antibody staining is too intense, the run should be repeated with the primary antibody incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
6. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
7. For corrective action, refer to the Step By Step Procedure section of the automated slide stainer Operator's Manual or contact your local Ventana office.

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