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

REF 790-4324 Σ 50

790-4325 Σ 250

IVD

INTENDED USE

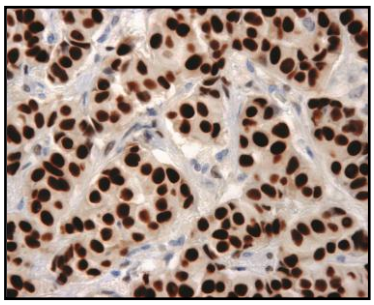


Figure 1. CONFIRM anti-Estrogen Receptor (ER) (SP1) staining of breast lobular carcinoma.

This antibody is intended for *in vitro* diagnostic (IVD) use.

CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody is intended for laboratory use for the qualitative detection of estrogen receptor (ER) antigen in sections of formalin-fixed, paraffin-embedded breast tissue on a VENTANA automated slide stainer with VENTANA detection kits and ancillary reagents. 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 hormone therapy for breast carcinoma.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

Prescription use 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 estrogen receptor (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]

Rabbit monoclonal antibodies have demonstrated improved sensitivity and specificity in immunohistochemistry.[3] Their reliability and staining quality is well established in breast carcinoma cases.[4,5] A large study of 4,150 invasive breast cancer cases demonstrated that anti-ER clone SP1 was a better prognostic factor than the mouse monoclonal clone 1D5.[6] Additionally, in a study utilizing 1,198 specimens of invasive breast carcinomas, SP1 was more sensitive in identifying ER expression in tumors than the mouse monoclonal clones 1D5 and 6F11.[7] Clinical use of anti-ER clone SP1 in a cohort of 508 cases revealed similar sensitivity when compared to mouse monoclonal antibodies but with more intense nuclear staining with SP1.[4] In another study, CONFIRM anti-ER (SP1) was used to determine semiquantitative hormone receptor values using a modified H-score based on percentage and intensity of staining. Subsequent quantitative measurement of ER receptors using RT-PCR in 80 breast cases demonstrated linear concordance when compared to CONFIRM anti-ER (SP1).[8]

Determination of ER status for all primary breast carcinomas was recommended by the National Institutes of Health (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. In 2010, the American Society of Clinical Oncology and the College of American Pathologists published a *Guideline for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer* and recommends that ER and PgR status be determined on all invasive breast cancers and breast cancer recurrences.[9] A number of methodologies to assess 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).[10] The immunohistochemical detection of ER has been described in cultured human breast cells,[11] some human breast cancer tissues,[11,12] human endometrium,[13] some endometrial cancers,[14] some low grade endometrial stromal sarcomas,[15] some cultured endometrial cells,[2] some sweat gland tumors,[16] some benign thyroid disease tissues,[17] some thyroid cancers,[18] some gastric cancers,[19,20] some prostatic carcinomas[21] and some female human bladders.[22]

Ventana Medical Systems, Inc. (Ventana) evaluated staining results with CONFIRM anti-ER (SP1) in normal tissues, neoplastic tissues, and 198 cases of breast carcinoma. 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).[23]

Breast cancer is the most common carcinoma occurring in women, and the second leading cause of cancer related death.[24] Early detection and appropriate treatment therapies can significantly affect overall survival.[25,26] 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, 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.[16]

A high ER concentration on the mammary tumor correlates with greater response to endocrine therapy.[18] 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).[25] Currently, the treatment of choice for ER positive carcinomas is tamoxifen.[25,26] Knowledge of ER status in breast tumors also aids in prognosis and treatment of the patient.[27] It has been shown in a number of studies that the presence of ER confers a favorable long term prognosis[25,28,29,30] and use of the SP1 clone specifically has been shown to have prognostic significance in patients who underwent hormone therapy.[7,31] If remission occurs, ER status must be reassessed, as it can change over time.[32]

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.[33] Other investigators, however, have found that lymph node metastases did not always maintain ER positivity.[32] 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. These tests utilizing tissues homogenates such as DCC or EIA may not be solely a reflection of ER status in malignant tissue.[34] 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.

REAGENT PROVIDED

Catalog Number 790-4324	CONFIRM anti-ER (SP1) contains sufficient reagent for 50 tests. One 5 mL dispenser of CONFIRM anti-ER (SP1) contains approximately 5 µg of a rabbit monoclonal antibody directed against human ER antigen.
Catalog Number 790-4325	CONFIRM anti-ER (SP1) contains sufficient reagent for 250 tests. One 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. There is trace (~0.2%) fetal calf serum of United States origin from the stock solution.

Total protein concentration of the reagent is approximately 20 mg/mL. Specific antibody concentration is approximately 1 µg/mL. There is no known non-specific antibody reactivity in this product.

CONFIRM anti-ER (SP1) is a rabbit monoclonal antibody produced as a cell culture supernatant.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as VENTANA detection kits (i.e., *ultra*View Universal DAB Detection Kit) and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser 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.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and the VENTANA BenchMark XT automated slide stainer or the VENTANA BenchMark ULTRA automated slide stainer. The following steps are recommended for processing specimens:[35]

1. Place specimen in 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).
2. After fixation, the specimen is placed in a tissue processing instrument for overnight preparation. Briefly, this processing consists of dehydration of the specimen with alcohols followed by clearing reagents to remove alcohols and finally infiltration with paraffin.
3. Samples are embedded with paraffin in tissue cassettes and approximately 4 µm thick sections are cut, centered and picked up on glass slides. The slides should be Superfrost Plus or equivalent. Tissue should be air dried by placing the slides at ambient temperature overnight or placed in a 60°C oven for 30 minutes.

Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative tissue controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. This product contains 1% or less bovine serum, which is used in the manufacture of the antibody.
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Avoid microbial contamination of reagents.
4. ProClin 300 is used as a preservative in this solution. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
5. Consult local or state authorities with regard to recommended method of disposal.
6. Refer to product Safety Data Sheet for additional information.

PRINCIPLE OF THE PROCEDURE

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 Universal DAB Detection Kit). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. 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 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.

Staining Procedure

VENTANA primary antibodies have been developed for use on the BenchMark XT or BenchMark ULTRA automated slide stainer in combination with VENTANA detection kits and accessories. Recommended staining protocols are listed below in Tables 1 and 2.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Verification and validation of the recommended staining procedure for each detection kit is demonstrated through design control testing and results of clinical studies.

Any modification to the recommended staining procedure nullifies the Performance Characteristics provided in this package insert. The user must validate any modification to the recommended staining procedure.

Table 1. Recommended Staining Protocols for CONFIRM anti-ER (SP1) using *ultra*View Universal DAB Detection Kit on a BenchMark XT and BenchMark ULTRA Instrument.

	Instrument/Method	
Procedure Type	**BenchMark XT Instrument**	**BenchMark ULTRA Instrument**
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard	Cell Conditioning 1, Standard
Enzyme (Protease)	None required	None required
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36°C
A/B Block (Biotin Blocking)	N/A	N/A
Counterstain (Hematoxylin)	Hematoxylin II, 4 minutes	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes	Bluing, 4 minutes

Table 2. Recommended Staining Protocols for CONFIRM anti-ER (SP1) using *i*VIEW DAB Detection Kit on a BenchMark XT and BenchMark ULTRA Instrument.

Procedure Type	Instrument/Method	
	BenchMark XT Instrument	BenchMark ULTRA Instrument
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard	Cell Conditioning 1, Standard
Enzyme (Protease)	None required	None required
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36°C
A/B Block (Biotin Blocking)	Required	Required
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 detailed instructions and additional protocol options, refer to your Operator's Manual.

BenchMark Automated IHC/ISH 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.
3. Check bulk fluids and waste.
4. Load the slides onto the automated slide stainer.
5. Start the staining run.
6. At the completion of the run, remove the slides from the automated slide stainer.
7. Wash in a mild dishwashing detergent or alcohol to remove the coverslip solution.
8. 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. The College of American Pathologists recommends that a positive tissue control should be on the patient slide.[9] 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 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. 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.

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 Antibody Diluent. Approximately 0.2% fetal calf serum is retained in the CONFIRM anti-ER (SP1). Addition of 0.2% fetal calf serum in 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 College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, or the CLSI Approved Guideline or both documents.[36] 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.

All quality control requirements should be performed in conformance with local, state and federal regulations or accreditation requirements.

STAINING INTERPRETATION

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. Estrogen receptor status is determined by the percentage of stained tumor cells. A case is considered ER positive if there is staining of the nucleus in equal to or greater than 1% of tumor cells. Specific staining of stroma and lymphocytes may be observed. It is imperative that only nuclear staining in tumor cells be considered when scoring these slides.

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., ≥1% in which nuclei of the tumor cells should be positive. 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. 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.[38]

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.[13] 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.[38] Contact your local support representative 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.[39]
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.[40]
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. 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.
3. This antibody is not intended for use in manual staining procedures.

PERFORMANCE CHARACTERISTICS

1. 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, intestine, 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.
2. Six individual tissues cases were stained as part of the repeatability testing. Of the six tissues, two had ER high expression, two ER low expression, and two ER negative based on a cutoff of <1% tumor cells staining for negative, 1-10% for low and >10% for high expression.

   For within-day repeatability (within-run) testing, 9 slides from each case were stained with CONFIRM anti-ER (SP1) antibody, and one slide from each case was stained with Negative Control Rabbit Ig antibody on a BenchMark XT instrument. The same testing configuration was also performed on a BenchMark ULTRA instrument. Within-day repeatability of CONFIRM anti-ER (SP1) antibody on both BenchMark XT and BenchMark ULTRA instruments was 100% concordant on all positive tissues across six cases. Negative Rabbit Control Ig stained slides were acceptable for signal and background.

   For day-to-day precision (between-run) testing, four slides from each case were stained with the CONFIRM anti-ER (SP1) antibody, and one slide from each case was stained with CONFIRM Negative Control Rabbit Ig antibody in five separate non-consecutive runs conducted over a 20 day period on the same BenchMark XT instrument. The same testing configuration was also performed on a BenchMark ULTRA instrument. Day-to-day precision of CONFIRM anti-ER (SP1) antibody on both BenchMark XT and BenchMark ULTRA instruments was 100% concordant on all positive tissues across six cases. Negative Rabbit Control IG stained slides were acceptable for signal and background.

For within-platform BenchMark XT instrument testing, 4 slides from six cases were stained with CONFIRM anti-ER (SP1) antibody across three separate BenchMark XT instruments. A single slide from each case was stained with Negative Control Rabbit Ig antibody. Instrument to instrument reproducibility of CONFIRM anti-ER (SP1) antibody on three BenchMark XT instruments was 100% concordant on all six cases. Negative Rabbit Control stained slides were acceptable for signal and background.

For within-platform BenchMark ULTRA instrument testing, 4 slides from six cases were stained with CONFIRM anti-ER (SP1) antibody across three separate BenchMark ULTRA instruments. A single slide from each case was stained with Negative Control Rabbit Ig antibody. Instrument to instrument reproducibility of CONFIRM anti-ER (SP1) antibody on three BenchMark ULTRA instruments was 100% concordant on all six cases. Negative Rabbit Control stained slides were acceptable for signal and background.

3. Comparison of BenchMark XT instrument versus BenchMark ULTRA instrument.

A randomized, multi-site, multi-reader study was conducted to compare the staining performance of the CONFIRM anti-ER (SP1) on the BenchMark ULTRA instrument versus the BenchMark XT instrument. One hundred twenty (120) ER negative and 132 ER positive cases of breast cancer, representing the clinical range of the assay, were randomly assigned to three study sites such that each site received an equal number of cases and each site received cases representing each clinical assessment category. Each site stained its assigned cases with the CONFIRM anti-ER (SP1) antibody on a BenchMark ULTRA instrument and a CONFIRM anti-ER (SP1) antibody on a BenchMark XT instrument. The stained slides were evaluated by pathologists who determined the percentage of stained tumor cells. A case was considered ER positive if there was staining of the nucleus in at least ≥1% of invasive tumor cells.[9]

Table 3. CONFIRM anti-ER (SP1) on the BenchMark ULTRA Instrument and CONFIRM anti-ER (SP1) on the BenchMark XT Instrument.

	BenchMark ULTRA Instrument		
BenchMark XT Instrument	**Positive**	**Negative**	**Total**
Positive	99	8	107
Negative	11	91	102
Total	110	99	209
	n/N	**% (95% CI)**	
Positive percent agreement	99/110	90.0 (83.0-94.3)	
Negative percent agreement	91/99	91.9 (84.9-95.8)	
Overall percent agreement	190/209	90.9 (86.2-94.1)	

The morphology acceptability rates for all slides stained in this study were 100% (95% C.I. 98.5%-100%) for the BenchMark ULTRA instrument and 94.0% (95% C.I. 90.4% - 96.4%) for the BenchMark XT instrument. The background acceptability rates were 94.8% (95% C.I. 91.4% - 97.0%) for the BenchMark ULTRA instrument and 90.9% (95% C.I. 86.7%-93.8%) for the BenchMark XT instrument.

4. Comparison of *i*VIEW DAB Detection Kit and *ultra*View Universal DAB Detection Kit using CONFIRM anti-ER (SP1).

CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody was used to conduct detection comparison testing across two instruments (BenchMark XT and BenchMark ULTRA instrument), using *i*VIEW DAB Detection Kit and *ultra*View Universal DAB Detection Kit. One hundred and ninety nine (199) tissue cases were used as part of the testing. Of the evaluable cases as determined by BenchMark ULTRA instrument, 111 were positive and 83 were negative as a function of percentage of tumor cells stained. The stained slides were evaluated by pathologists who determined the percentage of stained tumor cells. A case was considered ER positive if there was staining of the nucleus in at least 1% of tumor cells.

The morphology and background acceptability rates were 100% for both detection kits and instruments. Direct comparisons for positive and negative clinical assessment between detection kits, for each instrument are presented in Table 4 for the BenchMark ULTRA instrument and Table 5 for the BenchMark XT instrument.

Table 4. Assessment for *ultra*View Universal DAB Detection Kit versus *i*VIEW DAB Detection Kit on the BenchMark ULTRA Instrument.

***ultra*View Universal DAB Detection Kit**	***i*VIEW DAB Detection Kit**		
	Positive	**Negative**	**Total**
Positive	108	3	111
Negative	3	80	83
Total	111	83	194
	n/N	**% (95% CI)**	
Positive percent agreement	108/111	97.3 (92.4-99.1)	
Negative percent agreement	80/83	96.4 (89.9-98.8)	
Overall percent agreement	188/194	96.6 (93.4-98.6)	

Table 5. Assessment for *ultra*View Universal DAB Detection Kit versus *i*VIEW DAB Detection Kit on the BenchMark XT Instrument.

***ultra*View Universal DAB Detection Kit**	***i*VIEW DAB Detection Kit**		
	Positive	**Negative**	**Total**
Positive	106	5	111
Negative	2	79	81
Total	108	84	192
	n/N	**% (95% CI)**	
Positive percent agreement	106/108	98.1 (93.5-99.5)	
Negative percent agreement	79/84	94.0 (86.8-97.4)	
Overall percent agreement	185/192	96.4 (92.7-98.2)	

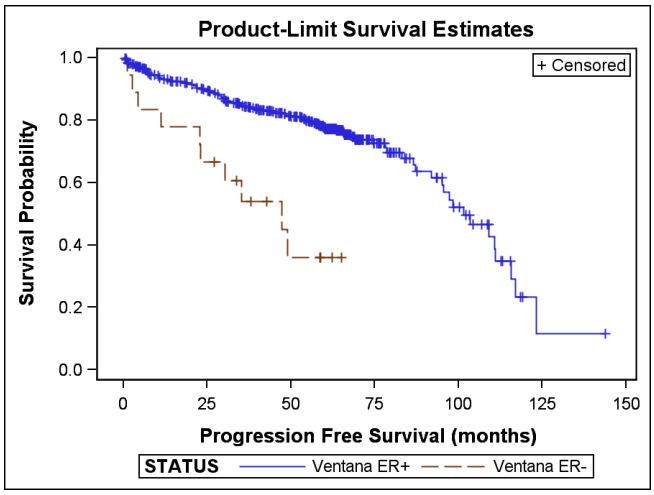
Overall agreements of assessment between detection kits for both platforms were 96.9% (n=194) and 96.4% (n=192) for the BenchMark ULTRA and the BenchMark XT instruments, respectively. The *ultra*View Universal DAB Detection Kit compared to *i*VIEW DAB Detection Kit had staining score agreement rates of 93.3% (n=194) and 93.8% (n=192).

5. Comparison to Patient Outcome.

A randomized, single-site, multi-reader study was conducted using a clinical cohort of 820 invasive breast cancer cases. Progression-free survival outcomes were compared for patients with different CONFIRM anti-ER (SP1) antibody status determined on the Benchmark ULTRA instrument. Cases were included in the analyses if the patient had a confirmed diagnosis of invasive breast carcinoma and received treatment with primary surgical intervention with or without post-operative local radiation therapy followed by adjuvant tamoxifen endocrine therapy (20 mg p.o./day) for 5 years. Cases were excluded from analyses if diagnostic biopsy or primary surgical tissue specimens were unavailable, if there had been a prior cancer diagnosis (except non-melanoma skin cancer), or if the patient received prior or adjuvant chemotherapy. A total of 1907 tissue microarray cores from 594 breast cancer cases with primary tumor were stained on the BenchMark ULTRA instrument. The stained slides were evaluated by three independent pathologists who determined the percentage of stained tumor cells. A case was considered ER positive if there was staining of the nucleus in at least ≥1% of invasive tumor cells.[9]

In the study, there were 441 patients with Ventana ER positive (ER+) status and 18 patients with Ventana ER negative (ER–) status. A Kaplan-Meier survival plot by CONFIRM anti-ER (SP1) status among the primary survival analysis population showed strong separation between Ventana ER+ and ER– cases. ER+ patients had longer survival times than ER– patients when tamoxifen treatment was administered; the median survival times for ER+ and ER– patients were 101.6 and 47.2 months, respectively. The log-rank test showed that the difference in survival plots was statistically significant (P < 0.001).

Figure 2. Kaplan-Meier Survival Plot by Ventana ER Status



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 support representative.

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