# CONFIRM anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal Primary Antibody

REF 790-2223 Σ 50

05277990001

REF 790-4296 Σ 250

05278392001

IVD

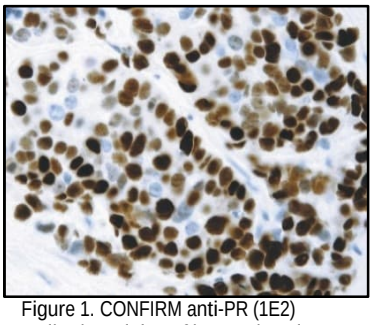

Figure 1. CONFIRM anti-PR (1E2) antibody staining of breast ductal carcinoma

## INTENDED USE

CONFIRM anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal (IgG) Primary Antibody is intended for laboratory use in the qualitative detection of progesterone receptor (PR) antigen in sections of formalin-fixed, paraffin-embedded tissue on a VENTANA automated slide stainer with VENTANA detection kits and ancillary reagents. CONFIRM anti-PR (1E2) is directed against an epitope present on human progesterone receptor protein located in the nucleus of PR positive normal and neoplastic cells.

CONFIRM anti-PR (1E2) 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.

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

## SUMMARY AND EXPLANATION

CONFIRM anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal Primary Antibody (CONFIRM anti-PR (1E2) antibody) is a rabbit monoclonal antibody that recognizes the A, and B isoforms of human progesterone receptor (PR). The immunogen was developed from a synthetic peptide identified as an area of potential high antigenicity common to progesterone receptor A and B forms. The peptide was synthesized and covalently bound to keyhole limpet hemocyanin to further increase antigenicity. CONFIRM anti-PR (1E2) antibody has been shown to react with 60 kD, 87 kD and 110 kD proteins from T47D cells via Western blotting. The protein sizes are in agreement with the predicted molecular weight of progesterone receptor forms A, B and C.[1]

PR is a nuclear hormone receptor encoded by a single gene (PGR).[1,2] PR activity is regulated by the closely related nuclear hormone receptor estrogen receptor (ER). The coordinated actions of ER and PR drive normal mammary gland development and are required for differentiation and proliferation in the adult breast epithelium.[1,2,3]

Breast cancer is the leading cause of cancer related death in women.[4] The diagnosis and treatment of the disease relies on early detection coupled with a reliable strategy to stratify patients into the appropriate therapy based on prognostic and predictive factors.[5,6] The established diagnostic workup of breast cancer is a combination of physical examination, imaging and pathological assessment.[5]

ER is one of the paradigm tumor markers for the management of breast cancer patients. Clinical guidelines and best practice recommendations stipulate that ER status should be evaluated in every case of primary invasive breast cancer to identify patients most likely to respond to endocrine forms of therapy.[5,6] Selective estrogen receptor modulators block estrogen mediated cancer growth by mitigating ER hyperactivity and are used as endocrine therapy for patients overexpressing the receptor.[6,7]

In practice, nearly half of ER positive patients fail to respond to endocrine treatment; a phenomenon linked to malignant transformation of the receptor in cancerous lesions.[8] PR overexpression can be used to further characterize the breast tumor and predict therapeutic response by acting as a reporter to evaluate the functional status of ER.[8,9] In 1975 it was hypothesized that the overexpression of PR can be a predictive marker for response to endocrine therapy.[8] Subsequent studies confirmed the predictive and prognostic value of PR.[10,11] A higher level of PR expression is an indicator of better response to endocrine therapy.[10]

The detection of PR is a cornerstone in the management of patients with invasive breast carcinoma.[5,6,9] Guidelines and best practice recommendations emphasize that immunohistochemistry (IHC) is the preferred method to detect PR in breast cancer.[9] Therefore, the IHC-based detection of PR with CONFIRM anti-PR (1E2) antibody may be used as an aid in the management, prognosis and prediction of therapy outcome of breast carcinoma.

## PRINCIPLE OF THE PROCEDURE

CONFIRM anti-PR (1E2) antibody binds to PR in formalin-fixed, paraffin embedded (FFPE) 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 Medical Systems, Inc. (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-PR (1E2) antibody, 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, a PR negative breast carcinoma) be included for every batch of samples processed and run on the BenchMark IHC/ISH instrument. This negative tissue control should be stained with CONFIRM anti-PR (1E2) antibody to ensure that the antigen enhancement and other pretreatment procedures did not create false positive staining.

## MATERIAL PROVIDED

CONFIRM anti-PR (1E2) antibody (Cat. No. 790-2223) contains sufficient reagent for 50 tests.

One 5 mL dispenser CONFIRM anti-PR (1E2) antibody contains approximately 5 µg of a rabbit monoclonal antibody directed against human PR antigen.

CONFIRM anti-PR (1E2) antibody (Cat. No. 790-4296) contains sufficient reagent for 250 tests.

One 25 mL dispenser CONFIRM anti-PR (1E2) antibody contains approximately 25 µg of a rabbit monoclonal antibody directed against human PR antigen.

The antibody is diluted in Tris-HCl with carrier protein, and 0.1% ProClin 300, a preservative. There is trace (~0.2%) fetal calf serum of U.S. origin from the stock solution.

Specific antibody concentration is approximately 1 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-PR (1E2) antibody is a rabbit monoclonal antibody produced as a cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

## MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

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

1. Recommended control tissue
2. Microscope slides, positively charged
3. CONFIRM Negative Control Rabbit Ig (Cat. No. 760-1029 / 05266238001)
4. *i*View DAB Detection Kit (Cat. No. 760-091 / 05266157001)

5. Endogenous Biotin Blocking Kit (Cat. No. 760-050 / 05266092001)
6. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
8. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Cell Conditioning 1 (CC1) (Cat. No. 950-124 / 05279801001)
12. ULTRA Cell Conditioning (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
14. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
15. BenchMark IHC/ISH instrument
16. General purpose laboratory equipment

## STORAGE AND STABILITY

Upon receipt and when not in use, 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 FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The following steps are recommended for processing of specimens.[12]

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).

After fixation specimen is placed in a tissue processing instrument for overnight preparation. Briefly, this processing consists of dehydration of specimen with alcohols followed by clearing reagents to remove alcohols and finally infiltration with paraffin.

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 immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of “Recommended Slide Storage and Handling” for more information.

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

## WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. ProClin 300 solution is used as a preservative in this reagent. 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. This product contains 1% or less bovine serum, which is used in the manufacture of the antibody.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[13,14]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and the instructions for use of all necessary components located navifyportal.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

| Hazard | Code | Statement |
|---|---|---|
| WARNING  | H317 | May cause an allergic skin reaction. |
| | H412 | Harmful to aquatic life with long lasting effects. |
| | P261 | Avoid breathing mist or vapours. |
| | P273 | Avoid release to the environment. |
| | P280 | Wear protective gloves. |
| | P333 + P313 | If skin irritation or rash occurs: Get medical advice/ attention. |
| | P362 + P364 | Take off contaminated clothing and wash it before reuse. |
| | P501 | Dispose of contents/ container to an approved waste disposal plant. |

This product contains CAS # 55965-84-9, a reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

## STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on a BenchMark IHC/ISH instrument in combination with VENTANA detection kits and accessories. Refer to Table 2 and Table 3 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4509 or 790-4296.

Verification and validation of the recommended staining protocol 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 method sheet. The user must validate any modification to the recommended staining procedure.

Table 2. Recommended staining protocol for CONFIRM anti-PR (1E2) antibody using *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

| Procedure Type | Method | |
|---|---|---|
| | XT | ULTRA or ULTRA PLUS |
| Deparaffinization | Selected | Selected |
| Cell Conditioning (Antigen Unmasking) | CC1, Standard | ULTRA CC1, Standard |
| Antibody (Primary) | 16 minutes, 37°C | 16 minutes, 36°C |
| Counterstain (Hematoxylin) | Hematoxylin II, 4 minutes | Hematoxylin II, 4 minutes |
| Post Counterstain | Bluing, 4 minutes | Bluing, 4 minutes |

Table 3. Recommended staining protocol for CONFIRM anti-PR (1E2) antibody using *i*VIEW DAB Detection Kit on BenchMark IHC/ISH instruments.

| Procedure Type | Method | |
|---|---|---|
| | XT | ULTRA |
| Deparaffinization | Selected | Selected |
| Cell Conditioning (Antigen Unmasking) | CC1, Standard | ULTRA CC1, Standard |
| Antibody (Primary) | 16 minutes, 37°C | 16 minutes, 36°C |
| A/B Block (Biotin Blocking) | Required | Required |
| Counterstain | Hematoxylin II, 4 minutes | Hematoxylin II, 4 minutes |
| Post Counterstain | Bluing, 4 minutes | Bluing, 4 minutes |

## QUALITY CONTROL PROCEDURES

### Positive Tissue Control

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

A positive tissue control must be run with every staining procedure performed. CAP 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-PR (1E2) antibody 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-PR (1E2) antibody 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.

### 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-PR (1E2) antibody for demonstration of PR, 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-PR (1E2) antibody 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 VENTANA Antibody Diluent. Approximately 0.2% fetal calf serum is retained in the CONFIRM anti-PR (1E2) antibody. 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 College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, or the CLSI Approved Guideline or both documents.[16,17] 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 / EXPECTED RESULTS

The staining procedure causes a colored reaction product to precipitate at the antigen sites localized by CONFIRM anti-PR (1E2) antibody. A qualified pathologist experienced in IHC procedures must evaluate positive and negative controls and qualify the stained product before interpreting results. Progesterone receptor status is determined by the percentage of stained tumor cells. A case is considered PR positive if there is staining of the nucleus in equal to or greater than 1% of tumor cells.[9]

### Positive Tissue Control

The positive tissue control stained with CONFIRM anti-PR (1E2) antibody 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%. Nuclei of the tumor cells should be positive. 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, PR 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. Certain stromal elements known to be PR negative such as endothelial cells 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.[18]

### Patient Tissue

Patient specimens stained with CONFIRM anti-PR (1E2) antibody should be examined last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. PR may be detected among other neoplasms, such as cancers of the ovary and endometrium.[19] 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. IHC 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.[20] 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.[21]
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.[18]
11. As with any IHC 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-PR (1E2) antibody negative result does not exclude the presence of PR. 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 estrogen receptor.
3. Positive nuclear staining with the antibody clone 1E2 has been seen in tonsil tissue.[22] Tonsil is not an indication for use with CONFIRM anti-PR (1E2) antibody. Therefore, if tonsil tissue is used as a negative tissue control, tonsil tissues should be screened to ensure a negative staining case is selected.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

## PERFORMANCE CHARACTERISTICS

### ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

### Sensitivity and Specificity

Sensitivity and Specificity of CONFIRM anti-PR (1E2) antibody was determined by staining multiple cases of normal human tissues. The results are listed in Table 4 and Table 5. Positive staining was nuclear in all tissues examined with one case of ovary showing unexpected negative staining. Positive staining of thyroid tissue was observed, but this has been identified previously.[23] Positive nuclear staining with the antibody clone 1E2 has been seen in tonsil tissue.[22]

Table 4. Sensitivity/Specificity of CONFIRM anti-PR (1E2) antibody was determined by testing FFPE normal tissues.

| Tissue | # positive / total cases | Tissue | # positive / total cases |
|---|---|---|---|
| Cerebrum | 0/5 | Esophagus | 0/3 |
| Cerebellum | 0/3 | Stomach | 0/3 |
| Adrenal gland | 0/3 | Small intestine | 0/3 |
| Ovary | 2/3 | Colon | 0/3 |
| Pancreas | 3/3 | Liver | 0/3 |
| Parathyroid gland | 0/4 | Salivary gland | 0/3 |
| Pituitary gland | 3/3 | Kidney | 0/3 |
| Testis | 0/3 | Prostate | 1/3 |
| Thyroid | 0/5 | Bladder | 0/5 |
| Breast[a] | 4/4 | Endometrium | 1/3 |
| Spleen | 1/3 | Cervix | 8/8 |
| Tonsil | 1/3 | Skeletal muscle | 0/5 |
| Thymus | 0/3 | Skin | 0/3 |
| Bone marrow | 0/3 | Nerve | 0/3 |
| Lung | 0/3 | Mesothelium | 0/3 |

| Tissue | # positive / total cases | Tissue | # positive / total cases |
|---|---|---|---|
| Heart | 0/3 | | |

[a] Tissue include fibrofatty tissue.

Immunoreactivity of CONFIRM anti-PR (1E2) antibody was determined by staining multiple cases of neoplastic human tissues. Cases were considered PR positive if there was staining of the nucleus in at least ≥ 1% of invasive tumor cells.

Table 5. Sensitivity/Specificity of CONFIRM anti-PR (1E2) antibody was determined by testing a variety of FFPE neoplastic tissues.

| Pathology | # positive / total cases |
|---|---|
| Glioblastoma (Cerebrum) | 0/1 |
| Meningioma (Cerebrum) | 0/1 |
| Ependymoma (Cerebrum) | 0/1 |
| Oligodendroglioma (Cerebellum) | 0/1 |
| Serous adenocarcinoma (Ovary) | 1/1 |
| Mucinous adenocarcinoma (Ovary) | 0/1 |
| Neuroendocrine neoplasm (Pancreas) | 1/1 |
| Adenocarcinoma (Pancreas) | 0/1 |
| Seminoma (Testis) | 0/1 |
| Embryonal carcinoma (Testis) | 0/1 |
| Medullary carcinoma (Thyroid) | 1/1 |
| Papillary carcinoma (Thyroid) | 0/1 |
| Ductal carcinoma in situ (Breast) | 1/1 |
| Invasive ductal carcinoma (Breast) | 0/1 |
| B-Cell Lymphoma; NOS (Spleen) | 0/1 |
| Small cell carcinoma (Lung) | 0/1 |
| Squamous cell carcinoma (Lung) | 0/1 |
| Adenocarcinoma (Lung) | 0/1 |
| Squamous cell carcinoma (Esophagus) | 0/1 |
| Adenocarcinoma (Esophagus) | 0/1 |
| Mucinous adenocarcinoma (Stomach) | 0/1 |
| Adenocarcinoma (Intestine) | 0/1 |
| Adenocarcinoma (Colon) | 0/1 |
| Malignant mixed mesenchymal neoplasm (Colon) | 0/1 |
| Adenocarcinoma (Rectum) | 0/1 |
| Malignant mixed mesenchymal neoplasm (Rectum) | 0/1 |
| Melanoma (Rectum) | 0/1 |
| Hepatocellular carcinoma (Liver) | 0/1 |
| Hepatoblastoma (Liver) | 0/1 |
| Clear cell carcinoma (Kidney) | 0/1 |
| Adenocarcinoma (Prostate) | 1/1 |
| Urothelial carcinoma (Prostatic urethra) | 1/1 |
| Leiomyoma (Uterus) | 1/1 |
| Adenocarcinoma (Uterus) | 1/2 |
| Clear cell carcinoma (Uterus) | 0/1 |
| Squamous cell carcinoma (Cervix) | 1/1 |
| Embryonal rhabdomyosarcoma (Striated muscle) | 0/1 |
| Squamous cell carcinoma (Striated muscle) | 0/1 |
| Basal cell carcinoma (Skin) | 0/1 |
| Neurofibroma (Mediastinum) | 1/1 |
| Neuroblastoma (Retroperitoneum) | 1/1 |
| Mesothelioma (Peritoneum) | 0/1 |
| Hodgkin lymphoma (Lymph node) | 0/1 |
| Lymphoma, NOS (Lymph node) | 0/2 |
| B-cell lymphoma, NOS (Lymph node) | 0/1 |
| Urothelial carcinoma (Bladder) | 0/1 |

Sensitivity is dependent upon the preservation of the antigen. Any improper tissue handling during fixation, sectioning, embedding or storage which alters antigenicity weakens PR detection by CONFIRM anti-PR (1E2) antibody and may generate false negative results.

### BenchMark XT and BenchMark ULTRA Instrument Precision

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

For within-run repeatability testing, 9 slides from each case were stained with CONFIRM anti-PR (1E2) antibody, and one slide from each case was stained with CONFIRM Negative Control Rabbit Ig antibody on a BenchMark XT instrument. The same testing configuration was also performed on a BenchMark ULTRA instrument. Within-run repeatability of CONFIRM anti-PR (1E2) antibody on both BenchMark XT and BenchMark ULTRA instruments was 100% concordant on all positive tissues across six cases.

For between-day intermediate precision testing, four slides from each case were stained with the CONFIRM anti-PR (1E2) 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. Between-day intermediate precision of CONFIRM anti-PR (1E2) antibody on both BenchMark XT and BenchMark ULTRA instruments was 100% concordant on all positive tissues across six cases.

For BenchMark XT between-instrument intermediate precision testing, 4 slides from six cases were stained with CONFIRM anti-PR (1E2) antibody across three separate BenchMark XT instruments. A single slide from each case was stained with CONFIRM Negative Control Rabbit Ig antibody. Between instrument intermediate precision of CONFIRM anti-PR (1E2) antibody on three BenchMark XT instruments was 100% concordant on all six cases.

For BenchMark ULTRA between-instrument intermediate precision testing, 4 slides from six cases were stained with CONFIRM anti-PR (1E2) antibody across three separate BenchMark ULTRA instruments. A single slide from each case was stained with CONFIRM Negative Control Rabbit Ig antibody. Between-instrument intermediate precision of CONFIRM anti-PR (1E2) antibody on three BenchMark ULTRA instruments was 100% concordant on all six cases.

All reproducibility testing met the acceptance criteria for passing.

### BenchMark ULTRA PLUS Instrument Precision

Nine individual tissue cases were stained as part of the precision testing. Of the nine tissues, three had PR high expression, three had PR low expression, and three were PR negative based on a cutoff of < 1% tumor cells staining for negative, 1-10% for low and > 10% for high expression.

For within-run repeatability testing, five slides from each case were stained with CONFIRM anti-PR (1E2) antibody, and one slide from each case was stained with CONFIRM

Negative Control Rabbit Ig antibody on a BenchMark ULTRA PLUS instrument. Within-run repeatability of CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA PLUS instrument was 100% concordant. CONFIRM Negative Control Rabbit Ig stained slides were acceptable for signal and background.

For between-day intermediate precision testing, two slides from each case were stained with the CONFIRM anti-PR (1E2) 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 ULTRA PLUS instrument. Between-day intermediate precision of CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA PLUS instrument was 97.8% concordant. CONFIRM Negative Control Rabbit Ig stained slides were acceptable for signal and background.

For BenchMark ULTRA PLUS between-instrument intermediate precision testing, two slides from each case were stained with CONFIRM anti-PR (1E2) antibody across three separate BenchMark ULTRA PLUS instruments. A single slide from each case was stained with CONFIRM Negative Control Rabbit Ig antibody. Between-instrument intermediate precision of CONFIRM anti-PR (1E2) antibody on three BenchMark ULTRA PLUS instruments was 98.1%.

### Inter-Laboratory Reproducibility

An Inter-Laboratory Reproducibility study for CONFIRM anti-PR (1E2) antibody was conducted using 14 breast cancer slides (8 positive, 2 low positive, 4 negative) run across 3 BenchMark XT and 3 BenchMark ULTRA instruments, using *i*VIEW DAB detection and *ultra*View Universal DAB Detection Kit, on each of 5 non-consecutive days over a minimum 20 day period at 3 external laboratories. The specimens were randomized and evaluated by a total of 6 pathologists (2 pathologists per site) for percentage of stained tumor cells. A case was considered PR positive if there was staining of the nucleus in at least 1% of invasive tumor cells.[9]

For Site-to-Site precision, the average positive agreement (APA) and average negative agreement (ANA) rates for CONFIRM anti-PR (1E2) antibody clinical assessment were 99.7% and 99.1%, respectively, on the BenchMark ULTRA instrument with *i*VIEW detection; 98.6% and 95.4%, respectively, on the BenchMark ULTRA instrument with *ultra*View Universal DAB Detection Kit; 99.4% and 98.4%, respectively, on the BenchMark XT instrument with *i*VIEW detection; and 97.4% and 91.3%, respectively, on the BenchMark XT instrument with *ultra*View Universal DAB Detection Kit.

For between-day precision, the APA and ANA rates for CONFIRM anti-PR (1E2) antibody clinical assessment were 99.7% and 99.1%, respectively, on the BenchMark ULTRA instrument with *i*VIEW detection; 98.5% and 95.5%, respectively, on the BenchMark ULTRA instrument with *ultra*View Universal DAB Detection Kit; 99.3% and 98.3%, respectively, on the BenchMark XT instrument with *i*VIEW detection; and 97.0% and 90.8%, respectively, on the BenchMark XT instrument with *ultra*View Universal DAB Detection Kit.

For Reader-to-Reader precision, the APA and ANA for CONFIRM anti-PR (1E2) antibody clinical assessment were 99.7% and 99.1%, respectively, on the BenchMark ULTRA instrument with *i*VIEW detection; 98.6% and 95.6%, respectively, on the BenchMark ULTRA instrument with *ultra*View Universal DAB Detection Kit; 99.3% and 98.3%, respectively, on the BenchMark XT instrument with *i*VIEW detection; and 98.6% and 95.1%, respectively, on the BenchMark XT instrument with *ultra*View Universal DAB Detection Kit.

For between-platform precision across the BenchMark ULTRA and BenchMark XT instruments, APA and ANA rates were 99.5% and 98.7%, respectively, for *i*VIEW detection, and 98.0% and 93.9%, respectively, for *ultra*View Universal DAB Detection Kit.

For within-platform precision, APA and ANA rates were 98.7% and 96.4%, respectively, for the BenchMark ULTRA instrument and 97.4% and 92.6%, respectively, for the BenchMark XT instrument.

### Comparison of *i*VIEW DAB Detection Kit and *ultra*View Universal DAB Detection Kit using CONFIRM anti-PR (1E2) antibody.

CONFIRM anti-PR (1E2) antibody was used to conduct detection comparison testing across two instruments (BenchMark XT and BenchMark ULTRA instruments) using *i*VIEW DAB Detection Kit and *ultra*View Universal DAB Detection Kit. One hundred and ninety-nine tissue cases were used as part of the testing. Of the cases, approximately half are positive and half 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 PR 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 except for *ultra*View Universal DAB Detection Kit on the BenchMark ULTRA instrument, which had a background acceptability rate of 99.5%. Direct comparisons for positive and negative clinical assessment between detection kits for each platform are presented in Table 6 for the BenchMark ULTRA instrument and Table 7 for the BenchMark XT instrument.

Table 6. Clinical assessment for *ultra*View Universal DAB Detection Kit vs. *i*VIEW DAB Detection Kit with the BenchMark ULTRA Instrument.

| ***ultra***View Universal DAB Detection Kit | *i*VIEW DAB Detection Kit | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 94 | 11 | 105 |
| Negative | 2 | 86 | 88 |
| Total | 96 | 97 | 193 |
| | n/N | % (95% CI) | |
| Positive percent agreement | 94/96 | 97.9 (92.7-99.4) | |
| Negative percent agreement | 86/97 | 88.7 (80.8-93.5) | |
| Overall percent agreement | 180/193 | 93.3 (88.8-96.0) | |

Table 7. Clinical assessment for *ultra*View Universal DAB Detection Kit vs. *i*VIEW DAB Detection Kit with the BenchMark XT Instrument.

| ***ultra***View Universal DAB Detection Kit | *i*VIEW DAB Detection Kit | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 91 | 14 | 105 |
| Negative | 2 | 86 | 88 |
| Total | 93 | 100 | 193 |
| | n/N | % (95% CI) | |
| Positive percent agreement | 91/93 | 97.8 (92.5-99.4) | |
| Negative percent agreement | 86/100 | 86.0 (77.9-91.5) | |
| Overall percent agreement | 177/193 | 91.7 (87.0-94.8) | |

Agreement of clinical assessment between detection kits for both instruments was above 90% at 93.3% (n=193) and 91.7% (n=193) for BenchMark ULTRA and BenchMark XT instruments respectively. The *ultra*View Universal DAB Detection Kit compared to *i*VIEW DAB Detection Kit had staining score agreement rates of 90.2% (n=193) and 85.5% (n=193).

### Comparison of BenchMark ULTRA Instrument versus BenchMark ULTRA PLUS Instrument

A study was conducted to compare the staining performance of the CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA PLUS instrument versus the BenchMark ULTRA instrument. One hundred thirty-four (134) breast carcinoma tissue cases (61 PR positive, 61 PR negative and 12 PR borderline positive), representing the clinical range of the assay. The stained slides were evaluated by pathologists who determined the percentage of stained tumor cells. A case was considered PR positive if there was staining of the nucleus in at least ≥ 1% of invasive tumor cells.[9] The direct comparison for positive and negative PR status between the BenchMark ULTRA instrument and the BenchMark ULTRA PLUS instrument is presented in Table 8.

Table 8. CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA PLUS instrument and CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA instrument (excluding borderline positive cases).

| BenchMark ULTRA PLUS instrument | BenchMark ULTRA instrument | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 79 | 2 | 81 |
| Negative | 6 | 23 | 29 |

| BenchMark ULTRA PLUS instrument | BenchMark ULTRA instrument | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Total | 85 | 25 | 110 |
| | n/N | % (95% CI) | |
| Positive percent agreement | 79/85 | 92.9 (85.4-96.7) | |
| Negative percent agreement | 23/25 | 92.0 (75.0-97.8) | |
| Overall percent agreement | 102/110 | 92.7 (86.3-96.3) | |

The morphology acceptability rate for all slides stained in this study was 100.0% (95% C.I. 97.2%-100.0%) for the BenchMark ULTRA PLUS instrument. The background acceptability rate was 100.0% (95% C.I. 97.2%-100.0%) for the BenchMark ULTRA PLUS instrument.

## CLINICAL PERFORMANCE

### Comparison of CONFIRM anti-PR (1E2) antibody to FLEX anti-PR (PgR 636).

A randomized, multi-site, multi-reader study was conducted to compare the staining performance of the CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA instrument and on the BenchMark XT instrument to that of the Dako FLEX Monoclonal Mouse Anti-Human Progesterone Receptor Clone PgR 636 Ready-To-Use (FLEX anti-PR (PgR 636)) on the Dako Autostainer Plus. On the BenchMark IHC/ISH instruments, endogenous biotin was blocked using the VENTANA Endogenous Biotin Blocking Kit. The antibody was detected using *i*VIEW DAB Detection Kit. On the Dako platform, the antibody was detected using EnVision Flex, High pH detection. Approximately 120 negative and 216 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 allotted cases with the CONFIRM anti-PR (1E2) antibody on a BenchMark ULTRA instrument, CONFIRM anti-PR (1E2) antibody on a BenchMark XT instrument, and with the FLEX anti-PR (PgR 636) on a Dako Autostainer Plus. The stained slides were evaluated by pathologists who determined the percentage of stained tumor cells. A case was considered PR positive if there was staining of the nucleus in at least ≥ 1% of invasive tumor cells.[9]

Table 9. CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA Instrument Compared to FLEX anti-PR (PgR 636) on the Dako Autostainer Plus.

| CONFIRM anti-PR (1E2) antibody | FLEX anti-PR (PgR 636) | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive: | 200 | 7 | 207 |
| Negative: | 9 | 104 | 113 |
| Total: | 209 | 111 | 320 |
| | n/N | % (95% CI) | |
| Positive percent agreement | 200/209 | 95.7 (92.0-97.7) | |
| Negative percent agreement | 104/111 | 93.7 (87.6-96.9) | |
| Overall percent agreement | 304/320 | 95.0 (92.0-96.9) | |

Table 10. CONFIRM anti-PR (1E2) antibody on the BenchMark XT Instrument Compared to FLEX anti-PR (PgR 636) on the Dako Autostainer Plus.

| CONFIRM anti-PR (1E2) antibody | FLEX anti-PR (PgR 636) | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive: | 186 | 9 | 195 |
| Negative: | 18 | 100 | 118 |
| Total: | 204 | 109 | 313 |
| | n/N | % (95% CI) | |
| Positive percent agreement | 186/204 | 91.2 (86.5-94.3) | |
| Negative percent agreement | 100/109 | 91.7 (85.0-95.6) | |
| Overall percent agreement | 286/313 | 91.4 (87.7-94.0) | |

For CONFIRM anti-PR (1E2) antibody staining on the BenchMark ULTRA and BenchMark XT instruments compared to FLEX anti-PR (PgR 636) on the Dako Autostainer Plus, the positive, negative, and overall agreement rates (pooled across all sites) were all greater than 90%.

Table 11. CONFIRM anti-PR (1E2) antibody on the BenchMark ULTRA Instrument Compared to CONFIRM anti-PR (1E2) antibody on the BenchMark XT Instrument.

| BenchMark ULTRA Instrument | BenchMark XT Instrument | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive: | 184 | 12 | 196 |
| Negative: | 6 | 105 | 111 |
| Total: | 190 | 117 | 307 |
| | n/N | % (95% CI) | |
| Positive percent agreement | 184/190 | 96.8 (93.3-98.5) | |
| Negative percent agreement | 105/117 | 89.7 (82.9-94.0) | |
| Overall percent agreement | 289/307 | 94.4 (90.9-96.3) | |

For CONFIRM anti-PR (1E2) antibody staining on the BenchMark ULTRA instrument compared to the BenchMark XT instrument, the positive, negative, and overall agreement rates were all greater than 89%.

The morphology acceptability rates for all slides stained in this study were 99.7% (95% C.I. 98.3%-99.9%) on the BenchMark ULTRA instrument and 96.1% (95% C.I. 93.5%-97.7%) on the BenchMark XT instrument. The background acceptability rates were 99.4% (95% C.I. 97.9%-99.8%) on the BenchMark ULTRA instrument and 95.2% (95% C.I. 92.4%-97.0%) on the BenchMark XT instrument.

## TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, check the other positive controls run concurrently to determine if it is due to the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, check to ensure that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls run concurrently 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. Follow proper procedure 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, repeat the deparaffinization procedure.
5. If specific antibody staining is too intense, repeat the run with the primary antibody incubation time shortened by 4 minute intervals to achieve the desired stain intensity.
6. If tissue sections wash off the slide, check slides to ensure that they are positively charged.
7. For corrective action, refer to the Step By Step Procedure section of the instrument User Guide or contact your local support representative.

## REFERENCES


1. Kariagina A, Aupperlee MD, Haslam SZ. Progesterone receptor isoform functions in normal breast development and breast cancer. Crit Rev Eukaryot Gene Expr. 2008;18(1):11-33.
2. Grimm SL, Hartig SM, Edwards DP. Progesterone Receptor Signaling Mechanisms. J Mol Biol. 2016;428(19):3831-3849.
3. Tanos T, Rojo L, Echeverria P, et al. ER and PR signaling nodes during mammary gland development. Breast Cancer Res. 2012;14(4):210.
4. Torre LA, Islami F, Siegel RL, et al. Global Cancer in Women: Burden and Trends. Cancer Epidemiol Biomarkers Prev. 2017;26(4):444-457.
5. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2019.
6. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013;24(9):2206-2223.
7. Swaby RF, Sharma CG, Jordan VC. SERMs for the treatment and prevention of breast cancer. Rev Endocr Metab Disord. 2007;8(3):229-239.
8. Horwitz KB, McGuire WL. Predicting response to endocrine therapy in human breast cancer: a hypothesis. Science. 1975;189(4204):726-727.
9. Hammond ME, Hayes DF, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med. 2010;134(6):907-22.
10. Bardou VJ, Arpino G, Elledge RM, et al. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol. 2003;21(10):1973-1979.
11. Ravdin PM, Green S, Dorr TM, et al. Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Group study. J Clin Oncol. 1992;10(8):1284-1291.
12. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
13. Occupational Safety and Health Standard: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
14. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.
15. Roche PC, His ED. Immunhistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
16. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2010.
17. CLSI. Quality Assurance for Immunocytochemistry: Approved Guideline. CLSI document MM4-A- (ISBN 1-56238-396-5). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.
18. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med. 1983;14:767.
19. Press MF, Greene GL. Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor. Endocrinology. 1988;122(3):1165-1175.
20. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66(4):194-199.
21. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol. 1980;73(5):626-32.
22. Guadagno E, De Rosa G, Nappi O. A National Quality Assurance Program for Breast Immunohistochemistry: An Italian Perspective. Pathologica. 2018;110(2):83-91.
23. Money SR, Muss W, Thelmo WL, et al. Immunocytochemical localization of estrogen and progesterone receptors in human thyroid. Surgery. 1989;106(6):975-8.


NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

## Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

| | |
|---|---|
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

## REVISION HISTORY

| Rev | Updates |
|---|---|
| H | Correction to Table 8 reference. Updated to current template. |

## INTELLECTUAL PROPERTY



For USA: Rx only

## CONTACT INFORMATION


Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, AZ 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany
+800 5505 6606


CE 0123