VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody

REF 790-4493

05999570001

IVD Σ 50

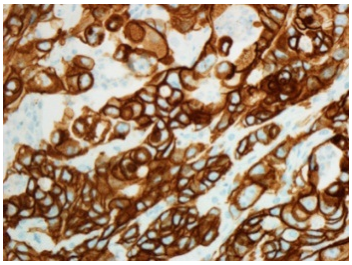

Figure 1. VENTANA anti-HER2/neu (4B5) 3+ staining in gastric carcinoma.

INTENDED USE

VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody (VENTANA HER2 (4B5)) is intended for the semi-quantitative detection of HER2 antigen in sections of formalin-fixed, paraffin-embedded normal and neoplastic breast and gastric tissue on a VENTANA automated IHC/ISH slide staining device.

It is indicated as an aid in the assessment of breast and gastric cancer patients for whom Herceptin treatment is considered and for breast cancer patients for whom Kadcyla (trastuzumab emtansine) or Perjeta (pertuzumab) treatments are being considered.

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

This antibody is intended for *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

VENTANA HER2 (4B5) is a rabbit monoclonal antibody (clone 4B5) directed against the internal domain of the c-erbB-2 oncoprotein (HER2). c-erbB-2 oncoprotein was cloned and characterized by Akiyama et al in 1986.[1] It is an approximately 185 kD transmembrane glycoprotein, which is structurally similar to epidermal growth factor receptor (EGFR). The protein is associated with tyrosine kinase activity similar to that of several growth factor receptors, and to that of the transforming proteins of the *src* family. The coding sequence is consistent with an extracellular binding domain and an intracellular kinase domain. This suggests that HER2 may be involved in signal transduction and stimulation of mitogenic activity.[1]

Clone 4B5 has been shown to react with a 185 kD protein from SK-BR-3 cell lysates via Western blotting. SK-BR-3 is a breast carcinoma cell line which has a 128 fold over expression of HER2 mRNA.[2] The size of the band identified correlates well with that reported for HER2 protein (185 kD).[1] Immunohistochemistry experiments with transfected cell lines (HEK293) have shown that clone 4B5 stains cells transfected with HER2 and cells transfected with HER4. No staining of cells transfected with HER1 or HER3 was observed. Western blot data with recombinant HER4 protein also indicated that clone 4B5 recognizes a HER4 epitope.

In breast carcinoma the HER2 protein is expressed at a level detectable by immunohistochemistry in up to 20% of adenocarcinomas from various sites. Between 15-30% of invasive ductal cancers are positive for HER2.[3] Almost all cases of Paget's disease of breast[4] and up to 90% of cases of ductal carcinoma *in situ* of comedo type are positive.[3] In gastric carcinoma the HER2 protein is expressed at a level detectable by immunohistochemistry in up to 30% of intestinal type, 15% mixed type and 5% diffuse type gastric cancers. The immunohistochemical detection of HER2 protein overexpression is also used as an aid in determination of patients for whom HER2 targeted therapy is indicated.[5,31-35]

Staining results in normal tissues, neoplastic tissues, and 322 cases of breast carcinoma with clone 4B5 were evaluated by Ventana. In the normal tissues tested, expression was consistent with the published literature in that there was no unexpected specific cytoplasmic/membrane staining, with the following exceptions: two cases of tonsil showing with epithelial cell membrane staining, one case of parathyroid, and one case of esophageal epithelium. Of the neoplastic tissues tested, cytoplasmic/membrane staining was seen in cancer cells of the breast, colon and ovary. Three hundred twenty-two (322) breast carcinomas were evaluated with clone 4B5 in a method comparison study with PATHWAY HER-2/neu (CB11). There is a significant correlation of staining between these two tests. See Summary of Expected Results section for further information. Additional information on clone 4B5 can be found in the References.[24-30]

The use of pre-diluted VENTANA HER2 (4B5) and ready-to-use *i*VIEW DAB Detection Kit and *ultra*View Universal DAB Detection Kit, in combination with a VENTANA automated IHC/ISH slide stainer, reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting, and manual reagent application.

CLINICAL SIGNIFICANCE

Breast cancer is the most common carcinoma occurring in women, and the second leading cause of cancer related death. In North America, a woman's chance of contracting breast cancer is one in eight.[6] Early detection and appropriate treatment therapies can significantly affect overall survival.[7] Gastric cancer is the fourth most common cancer and the second leading cause of cancer death globally. Surgery is the most common treatment for stomach cancer. However, most gastric cancer cases are detected at an advanced stage and the surgery is often difficult to perform. Chemotherapy is used for treating advanced gastric cancer even though the survival of cancer patients is very low. 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 their diagnosis and prognosis of disease. One important marker in breast and gastric cancer today is c-erbB-2 oncoprotein (HER2).

HER2 is a transmembrane protein.[8] It is closely related to EGFR and, like EGFR, has tyrosine kinase activity.[1] Gene amplification and the corresponding overexpression of c-erbB-2 has been found in a variety of tumors, including breast and gastric carcinomas.[8,9]

HER2 targeted therapies have been shown to benefit some breast and gastric carcinoma patients. Only patients with HER2 positive breast and gastric carcinomas should benefit from HER2 targeted treatment. *In vitro* diagnostics for the determination of HER2 status in breast and gastric carcinomas are important to aid the clinician in determination of patients eligible for treatment with HER2 targeted therapies.

Interpretation of the results of any detection system for HER2 must take into consideration the fact that HER2 is expressed in both breast and gastric cancer tumors and healthy tissue, albeit at differing levels and with different patterns of expression.[10] Histological tissue preparations have the advantage of intact tissue morphology to aid in the interpretation of the HER2 positivity of the sample.

PRINCIPLE OF THE PROCEDURE

VENTANA HER2 (4B5) is a rabbit monoclonal antibody, which binds to HER2 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. Each step is incubated for a precise time and temperature. At the end of each incubation step, the VENTANA automated IHC/ISH slide stainer washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies Liquid Coverslip, which minimizes evaporation of the aqueous reagents from the specimen slide.

Clinical cases should be evaluated within the context of the performance of appropriate controls. Ventana recommends the inclusion of a positive tissue control fixed and processed in the same manner as the patient specimen (for example, a weakly positive breast or gastric carcinoma). In addition to staining with VENTANA HER2 (4B5), 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 membrane staining of the tumor cells. 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 HER2 negative breast or gastric carcinoma) be included for every batch of samples processed and run on the VENTANA automated IHC/ISH slide stainer. This negative tissue control should be stained with VENTANA HER2 (4B5) to ensure that the antigen enhancement and other pretreatment procedures did not create false positive staining.

REAGENTS PROVIDED

VENTANA HER2 (4B5) dispenser contains sufficient reagent for 50 tests.

One 5 mL dispenser VENTANA HER2 (4B5) contains approximately 30 µg of a rabbit monoclonal antibody directed against human c-erbB-2 antigen.

The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative. There is trace fetal calf serum, approximately 0.25%, present from the stock solution.

Total protein concentration of the reagent is approximately 16 mg/mL. Specific antibody concentration is approximately 6 µg/mL. VENTANA HER2 (4B5) is a rabbit IgG diluted from tissue culture supernatants.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General 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 package insert may be available in all geographies. Consult your local support representative.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody after every run, the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and VENTANA BenchMark, BenchMark GX, BenchMark XT and BenchMark ULTRA automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.[2]

Approximately 4 µm thick sections should be cut and picked up on glass slides. The slides should be Superfrost Plus or equivalent. Studies at Ventana indicate that air dried cut tissue and cell line sections stored at 2-8°C are stable for at least 6 months. Each laboratory should validate the cut slide stability for their own procedures and environmental storage conditions.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. The use of this product for selection of patients eligible for Kadcyla or Perjeta therapies may not be available in all geographies. Please consult your local Roche representative to confirm availability in specific locations.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is sodium azide. Symptoms of overexposure to sodium azide include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of sodium azide in this product is 0.05% and does not meet the OSHA criteria for a hazardous substance. Buildup of NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide accumulation in plumbing.[12] Systemic allergic reactions are possible in sensitive individuals.
7. Consult local or state authorities with regard to recommended method of disposal.
8. Refer to the product Safety Data Sheet for additional information.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on a VENTANA BenchMark, BenchMark GX, BenchMark XT and BenchMark ULTRA automated slide stainer in combination with VENTANA detection kits and accessories. Refer to Table 1 and Table 2 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's Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for VENTANA HER2 (4B5) with *i*VIEW DAB Detection Kit on a BenchMark, BenchMark GX, BenchMark XT instrument and BenchMark ULTRA instrument.

	Method		
Procedure Type	BenchMark and BenchMark GX instrument	BenchMark XT instrument	BenchMark ULTRA instrument
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard	Cell Conditioning 1, Standard	ULTRA CC1, mild
Antibody (Primary)	32 minutes, 37°C	32 minutes, 37°C	24 minutes, 36°C
A/B Block (Biotin Blocking)	Not Selected	Selected	Selected
Counterstain (Hematoxylin)	Hematoxylin II, 4 minutes	Hematoxylin II, 4 minutes	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes	Bluing, 4 minutes	Bluing, 4 minutes

Table 2. Recommended Staining Protocol for VENTANA HER2 (4B5) with *ultra*View DAB Detection Kit on a BenchMark, BenchMark GX, BenchMark XT instrument and BenchMark ULTRA instrument.

	Method	
Procedure Type	BenchMark, BenchMark GX and BenchMark XT instrument	BenchMark ULTRA instrument
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild	ULTRA CC1, Mild
Antibody (Primary)	16 minutes, 37°C	12 minutes, 36°C
ultraWash	Selected	Selected
Counterstain	Hematoxylin II, 4 minutes	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes	Bluing, 4 minutes

QUALITY CONTROL PROCEDURES

Cell Line System Controls

Ventana has available as a separate product four formalin-fixed cell line controls embedded in paraffin, sectioned and placed on a single charged slide. PATHWAY HER-2 4 in 1 Control Slides (catalog # 781-2991) may be useful for a preliminary validation of the processing method used for staining slides with VENTANA HER2 (4B5). These four cell line controls are characterized by *in situ* hybridization for gene copy number. When processed and stained appropriately, the cell lines should stain as described in Table 4. If the indicated staining is not evident in the appropriate cores, especially the 1+ and 2+ controls, the staining of the tissues should be repeated.

Table 3. Characteristics of PATHWAY HER-2 4 in 1 Control Slides

HER2 IHC Score	Cell Line	HER2/Chr17 Ratio*
0	MDA-MB-231	1.11
1+	T47D	1.12
2+	MDA-MB-453	2.66
3+	BT-474	5.53

* HER2/Chr17 ratio is an average of three lots of PATHWAY HER-2 4 in 1 Control Slides determined using fluorescence *in situ* hybridization (FISH).

Positive Tissue Control

A positive control tissue fixed and processed in the same manner as the patient specimens must be run for each set of test conditions and with every VENTANA HER2 (4B5) staining procedure performed. This tissue could contain both positive staining cell/tissue components and negative cell tissue components and serve as both the positive and negative control tissue. Control tissue should be fresh autopsy/biopsy/surgical specimens prepared and fixed as soon as possible in a manner identical to test sections. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue preparation. A tissue with weak positive staining is more suitable than strong positive staining for optimal quality control and to detect minor levels of reagent degradation. Ideally a 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. Generally, however, neoplastic tissue that is positive for HER2 is strongly positive due to the nature of the pathology (overexpression). An example of a positive control for HER2 (4B5) is a known weak HER2 positive invasive breast carcinoma, or weakly positive gastric carcinoma specimen. The positive staining tissue components (membranous staining of neoplastic cells) are used to confirm that the antibody was applied and the instrument functioned properly.

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.

Negative Tissue Control

The same slide used for the positive tissue control (ductal or lobular invasive breast carcinoma, or gastric carcinoma) may be used as the negative tissue control. The non-staining components (surrounding stroma, lymphoid cells and blood vessels) should demonstrate absence of specific staining and provide an indication of specific background staining (false positive) with the primary antibody. Use a known negative tissue, fixed, processed and embedded in a manner identical to the patient sample(s).

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. The slide should be stained with CONFIRM Negative Control Rabbit Ig. The incubation period for the negative reagent control should equal the primary antibody incubation period.

Unexplained Discrepancies

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

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing it on a series of tissues with known immunohistochemistry performance characteristics representing known positive and negative tissues (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,[13] or the CLSI Approved Guideline[14] or both documents). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Breast and gastric cancer tissues with known HER2 status are suitable for assay verification.

INTERPRETATION OF RESULTS

The VENTANA automated immunostaining procedure causes a brown colored (DAB) reaction product to precipitate at the antigen sites localized by VENTANA HER2 (4B5). A qualified pathologist experienced in immunohistochemical procedures must evaluate controls and qualify the stained product before interpreting results.

Positive Controls

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the membrane of the target cells is indicative of positive reactivity. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

If the positive tissue control fails to demonstrate positive staining, any results with the test specimens should be considered invalid.

Negative Tissue Controls

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. If specific staining occurs in the negative tissue control, results with the patient specimen should be considered invalid.

Negative Reagent Controls

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 often stain nonspecifically.

Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. 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.

STAINING INTERPRETATION

Scoring Conventions for the Interpretation of VENTANA HER2 (4B5) in Breast Carcinoma

Breast carcinomas that are considered positive for HER2 protein overexpression must meet threshold criteria for intensity of staining (2+ or greater on a scale of 0 to 3+) and percent positive tumor cells (greater than 10%). Staining must also localize to the cellular membrane. Cytoplasmic staining may still be present, but this staining is not included in the determination of positivity. Scan complete tissue section to ensure scoring in well-preserved and well stained areas only. Staining that completely encircles the cytoplasmic membrane should be scored as an intensity of “2+” or “3+”. Partial staining of the membrane should be scored as a “1+”. It may be necessary to examine borderline cases at 40X or higher magnification to discriminate between intensities of “1+” and “2+”. In contrast to cases scored as an intensity of 3+, the staining scored as 2+ has a crisper and more clearly delineated ring, while cases scored as 3+ exhibit a very thick outline. Below is a quick reference chart for staining criteria. Refer to *Interpretation Guide for VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody staining of breast and gastric carcinoma* for a more detailed description with photographs of staining with VENTANA HER2 (4B5).

Table 4. Criteria for Intensity and Pattern of Cell Membrane Staining with VENTANA HER2 (4B5) in Breast Carcinoma.

Staining Pattern	Score (Report to Treating Physician)	HER2 Staining Assessment
No membrane staining is observed	0	Negative
Faint, partial staining of the membrane in any proportion of the cancer cells	1+	Negative
Weak complete staining of the membrane, greater than 10% of cancer cells	2+	Equivocal*
Intense complete staining of the membrane, greater than 10% of cancer cells	3+	Positive

*Recommend reflex to ISH

Scoring Conventions for the Interpretation of VENTANA HER2 (4B5) in Gastric Carcinoma

Gastric carcinomas that are considered positive for HER2 protein overexpression must meet a threshold criteria for the intensity and pattern of membrane staining (2+ or greater on a scale of 0 to 3+), and for the percent positive tumor cells. Staining must localize to the cell membrane but need not be completely circumferential, as baso-lateral staining is regularly observed and should be considered for scoring. Staining of the cytoplasm and/or the nucleus may be present, but this staining is not included in the determination of positivity. In gastric carcinoma the percentage of positive tumor cells depends upon whether the sample is a biopsy specimen (≥5 cohesive cells) or resection specimen (≥10%).

In establishing the scoring guidelines for HER2 immunohistochemistry in gastric cancer[15] note that while strong membranous staining is evidence of HER2 protein overexpression in neoplastic cells it need not be completely circumferential.

Rüschoff et al reported diffuse cytoplasmic staining with or without nuclear staining in gastric cancer.[16] Only membranous staining should be used in determination of HER2 protein expression in gastric cancer.

Immunohistochemical staining with the clone 4B5 can produce cytoplasmic and nuclear staining of normal gastric mucosa and more infrequently of neoplastic cells in gastric carcinoma and gastric/esophageal carcinoma. The nature of this cytoplasmic and nuclear staining is currently unknown. This staining pattern should not be confused with the discrete membranous staining, as that is indicative of HER2 positivity in neoplastic cells.

Refer to *Interpretation Guide for VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody staining of breast and gastric carcinoma* for a more detailed description with photomicrographs of staining with VENTANA HER2 (4B5).

Table 5. Criteria for Intensity and Pattern of Cell Membrane Staining with VENTANA HER2 (4B5) in Gastric Carcinoma.

Staining Pattern - Resection Specimen	Staining Pattern - Biopsy Specimen	Score (Report to requesting physician)	HER2 Staining Assessment
No reactivity or membranous reactivity in <10% of tumor cells	No reactivity or membranous reactivity in any tumor cell	0	Negative
Faint/barely perceptible membranous reactivity in ≥10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster* with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	1+	Negative
Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	2+	Equivocal**
Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	3+	Positive

*≥5 cohesive cells

** Recommend reflex to ISH

LIMITATIONS

General Limitations

1. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, 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 result from 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. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents and methods used to interpret 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.[17] 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.[18]
9. False positive results may be seen because of non-immunological 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.[19]
10. 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 has been optimized as indicated in tables 1 and 2 for VENTANA platforms and detection chemistries. Because of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[20]
2. 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.
3. Bone marrow was not tested for specificity. The user should determine appropriate staining in the above tissues prior to interpretation of staining information.
4. Immunohistochemical staining with clone 4B5 can produce cytoplasmic and nuclear staining of normal gastric mucosa and more infrequently of neoplastic cells in gastric carcinoma and gastric/esophageal carcinoma. The nature of this cytoplasmic and nuclear staining is currently unknown. This staining pattern should not be confused with the discrete membranous staining that is indicative of HER2 positivity in neoplastic cells.

PERFORMANCE CHARACTERISTICS

The performance of the VENTANA HER2 (4B5) antibody was evaluated through specificity, reproducibility and method comparison studies. All staining was performed using the *i*VIEW DAB Detection Kit protocol listed above on a Benchmark XT automated slide stainer unless otherwise specified. Data are presented first for breast carcinoma, and then for gastric carcinomas.

1. Specificity: Clone 4B5 specificity was determined by a study that showed no specific membrane staining for most normal tissues. Staining results were as follows: adrenal (0/3), breast (0/3), cerebellum (0/3), cerebrum (0/3), cervix (0/3), colon (0/3), esophagus (1/3), heart (0/2), kidney (0/3), liver (0/3), lung (0/3), mesothelial cells (0/3), ovary (0/3), pancreas (0/3), parathyroid (1/3, focal membrane staining), peripheral nerve (1/3), pituitary (0/2), prostate (1/3), salivary gland (0/3), skeletal muscle (0/3), skin (0/3), small intestine (0/3), spleen (0/3), stomach (0/3), testis (0/3), thymus (0/2), thyroid (0/3), tonsil (2/3 focal staining of surface epithelial cells), and uterus (0/3).

   Clone 4B5 specificity was also determined by a study that showed no specific membrane staining in most neoplastic tissues. Staining results were as follows: breast cancer (1/4), carcinoid (0/2), colon cancer (1/3), hepatocellular cancer (0/5), leiomyoma (0/2), lung cancer (0/2), lymphoma (0/3), melanoma (0/2), ovarian cancer (1/2), pancreatic cancer (0/3), prostate cancer (0/3), renal cell cancer (0/5), sarcoma (0/2), stomach cancer (0/3), thyroid cancer (0/3), and undifferentiated cancer (0/1).

   Positive staining in tonsilar epithelieum, esophageal epithelium, prostate, peripheral nerve, parathyroid, breast cancer, colon, and ovarian cancer are consistent with published literature regarding expression of HER2.
2. Sensitivity: Sensitivity is demonstrated in Tables 9 and 11: Consensus clone 4B5 IHC Scores of Three Pathologists Compared to FISH.
3. Intra-run reproducibility of staining on the BenchMark and BenchMark XT staining instrument platforms was determined by staining three slides each of five breast cancer tissues with a score of 0, 1+, 2+, and 3+ HER2 expression. For each case, three of 3 slides stained appropriately within a run and for all instrument platforms tested. Users should verify within run reproducibility results by staining several sets of serial sections with low, medium and high antigen density in a single run.
4. Inter-run and inter-platform reproducibility of staining was determined by staining three slides each of five breast cancer tissues with scores of 0, 1+, 2+, and 3+ HER2 expression on three different instrument runs across the BenchMark and BenchMark XT instrument platforms. For each case, nine of 9 slides stained appropriately over three instrument runs and across all instrument platforms tested.
5. BenchMark XT instrument inter-laboratory staining and inter-reader scoring reproducibility: Three laboratories participated in the inter-laboratory reproducibility study. Cut slides of 40 neutral buffered formalin-fixed invasive breast carcinoma cases [10 each from each HER2 binning category (0-1+, 2+, 3+)] and six (6) PATHWAY HER-2 4 in 1 Control Slides were shipped to each of the sites for staining on VENTANA BenchMark XT automated slide staining platform using the recommended staining protocol. Controls included the PATHWAY HER-2 4 in 1 Control Slides and a second slide of each case stained with negative Ig reagent. No sites experienced invalid runs, based upon the performance of the controls. The results were analyzed by Ventana. Thirty-four of forty (34/40) slides exhibited similar staining intensity across staining sites. Six samples (6/40 or 15%) varied by no more than 1 intensity level. Three (3/6) samples varied between 0 and 1+, which are both considered to be negative. Two samples (2/40 or 5%) varied between 2+ and 3+, and one sample (1/40) varied between 1+ and 2+.
6. BenchMark XT instrument inter-reader scoring reproducibility: In all of the 40 cases (100%), a minimum of 2 of 3 pathologists agreed.
7. Lot-to-lot reproducibility was determined by automated staining of 5 breast cancer tissues with scores of 0, 1+, 2+, and 3+ HER2 expression with 3 lots of HER2 (4B5). Stained tissues were scored on a 0 to 3+ scale by three qualified readers. There was 100% agreement between lots and readers for the 3 slides and 5 tissues stained.
8. Comparison studies of clone 4B5 rabbit monoclonal antibody to clone PATHWAY HER-2/neu (CB11) mouse monoclonal antibody: Summary of Studies Performed. A method comparison study was conducted to examine the correlation of clone 4B5 to PATHWAY HER-2/neu (CB11) and PathVysion Her-2 FISH, both previously approved FDA diagnostic tests. Six investigators participated in the study. Two sets of three different investigators evaluated two independent cohorts (Cohort 1: n=178, Cohort 2: n=144) using known breast cancer cases stained with PATHWAY HER-2/neu (CB11) and clone 4B5. FISH data was obtained from patient history. A consensus score from the three readers for each antibody was created for each case to reduce intra-reader variability known to exist with HER2 scoring.[21,22,23] A total of 322 cases were evaluated. The slides stained with PATHWAY HER-2/neu (CB11) were processed and stained according to the manufacturer's instructions specified in the PATHWAY HER-2/neu (CB11) package insert. There was an average of approximately one year between staining and reading of the PATHWAY HER-2/neu (CB11) stained slides. Since scores from one of the six readers was outside of the confidence interval (CI), data from the two cohorts are presented as follows:

Inter-pathologist Reproducibility of Comparison Studies Specimens

Table 6. Cohort 1: Consensus IHC Scores of Three Pathologists.

Clone 4B5 Score	PATHWAY HER-2/neu (CB11) Score			
	3+	2+	0, 1+	Total
3+	29	24	5	58
2+	2	13	17	32
0, 1+	0	0	53	53
Total	31	37	75	143

Cohort 1: Performance characteristics for 3 x 3 Presentation.

Overall agreement is 29+13+53/143=66.4% (95% CI = 38.6%, 59.7%).

Cohort 1: Performance characteristics for 2 x 2 Presentation (HER-2 antibody positive (2+ and 3+) and negative (0+ and 1+) scores are combined).

- Positive percent agreement is 29+2+24+13/31+37 =100% (95% CI= 97.5% - 100%).
- Negative percent agreement is 53/75 = 70.7% (95% CI = 58.5% - 80.1%).

Overall agreement is 29+24+2+13+53/143=84.7% (95% CI = 78.2% - 90.0).

Table 7. Cohort 2: Consensus IHC Scores of Three Pathologists.

Clone 4B5 Score	PATHWAY HER-2/neu (CB11) Score			
	3+	2+	0, 1+	Total
3+	72	1	0	73
2+	1	12	5	18
0, 1+	0	7	80	87
Total	73	20	85	178

Cohort 2: Performance characteristics for 3 x 3 Presentation.

Overall agreement is 72+12+80/178=92.1% (95% CI = 80.1%, 93.1%).

Cohort 2: Performance characteristics for 2 x 2 Presentation (HER2 antibody positive (2+ and 3+) and negative (0+ and 1+) scores are combined).

- Positive percent agreement is 72+12+1+1/73+20 = 92.5% (95% CI = 85.2% - 96.9%).
- Negative percent agreement is 80/85 = 94.1% (95% CI = 86.8% - 98.1%).

Overall agreement is 72+12+1+1+80/178=93.3% (95% CI = 88.5% - 96.4%).

Table 8. Cohort 1: Consensus PATHWAY HER-2/neu (CB11) IHC Scores of Three Pathologists Compared to FISH.

PATHWAY HER-2/neu (CB11) Score	FISH Result		
	Positive	Negative	Total
3+	32	0	32
2+	32	5	37
0, 1+	22	53	75
Total	86	58	144

Cohort 1: Performance characteristics for PATHWAY HER-2/NEU (CB11) and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive).

- Positive percent agreement is 32+32/ 86= 74.4% (95% CI = 63.8% - 83.2%).
- Negative percent agreement is 53/58 = 91.4% (95% CI = 80.9% - 97.1%).

Overall agreement is 32+32+53/144=81.2% (95% CI = 73.9% - 87.2%).

Table 9. Cohort 1: Consensus clone 4B5 IHC Scores of Three Pathologists Compared to FISH.

Clone 4B5 Score	FISH Result		
	Positive	Negative	Total
3+	55	3	58
2+	25	8	33
0, 1+	6	47	53
Total	86	58	144

Cohort 1: Performance characteristics for Clone 4B5 and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive).

- Positive percent agreement is 55+25/ 86= 93.0% (95% CI = 87.9% - 96.3%).
- Negative percent agreement is 47/58 = 81.0% (95% CI = 73.4% - 86.0%).

Overall agreement is 55+25+47/144=88.2% (95% CI = 82.1% - 92.2%).

Table 10. Cohort 2: Consensus PATHWAY HER-2/neu (CB11) IHC Scores of Three Pathologists Compared to FISH.

PATHWAY HER-2/neu (CB11) Score	FISH Result		
	Positive	Negative	Total
3+	72	1	73
2+	13	7	20
0, 1+	8	77	85
Total	93	85	178

Cohort 2: Performance characteristics for PATHWAY HER-2/neu (CB11) and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive).

- Positive percent agreement is 72+13/ 93= 91.3% (95% CI = 85.0% - 96.7%).
- Negative percent agreement is 77/85 = 90.6% (95% CI = 83.9% - 96.3%).

Overall agreement is 72+13+77/178=91.0% (95% CI = 86.5% - 94.9%).

Table 11. Cohort 2: Consensus clone 4B5 IHC Scores of Three Pathologists Compared to FISH.

Clone 4B5 Score	FISH Result		
	Positive	Negative	Total
3+	72	1	73
2+	11	7	18
0, 1+	10	77	87
Total	93	85	178

Cohort 2: Performance characteristics for Clone 4B5 and FISH, 2 x 2 Presentation (where scores of 2 and 3 are considered positive).

- Positive percent agreement is 72+11/ 93= 89.2% (95% CI = 82.5% - 95.1%).
- Negative percent agreement is 77/85 = 90.6% (95% CI = 84.0% - 96.4%).

Overall agreement is 72+11+77/178=90.0% (95% CI = 85.4% - 93.6%).

Inter-pathologist Reproducibility of Comparison Studies Specimens

Since it is well known that different pathologists may have different interpretations of immunohistochemistry slides, three pathologists were employed for each of the two cohorts (for a total of 6 pathologists) to read all samples. A two-out-of-three rule was used to adjudicate the final results. Below is a summary of the variable results obtained by the three pathologists of the comparison study samples for each cohort.

Table 12. Cohort 1: Clone 4B5 Scoring for the Three Pathologists.

HER2 Score	Clone 4B5 Score		
	Investigator 1	Investigator 2	Investigator 3
3+	72	70	73
2+	22	19	18
0,1+	80	89	87
Total	174	178	178

Note: A total of 3 samples varied by more than one grade level (i.e., 0, 2+) when evaluated by the three pathologists.

Sample 1: One pathologist scored 2+, two pathologists scored 0+.

Sample 2: One pathologist scored 0+ two pathologists scored 2+.

Sample 3: One pathologist scored 0+, the second scored 1+, and the third scored 2+.

Table 13. Cohort 1: PATHWAY HER-2/neu (CB11) Scoring for the Three Pathologists.

HER2 Score	PATHWAY HER-2/neu (CB11) Score		
	Investigator 1	Investigator 2	Investigator 3
3+	72	75	73
2+	22	22	18
0,1+	80	81	87
Total	174	178	178

Note: A total of 1 sample varied by more than one grade level (i.e., 1 - 3+) when evaluated by the three pathologists.

Sample 1: One pathologist scored 1+, the second scored 2+, and the third scored 3+.

Table 14. Cohort 2: Clone 4B5 Scoring for the Three Pathologists.

HER2 Score	Clone 4B5 Score		
	Investigator 4	Investigator 5	Investigator 6
3	59	65	50
2	30	28	39
0,1	52	51	55
Total	141	144	144

Note: A total of 6 samples varied by more than one grade level (e.g. 0, 3+) when evaluated by the three pathologists.

Sample 1: One pathologist scored 0+, the second scored 0+, and the third scored 2+.

Sample 2: One pathologist scored 1+, the second scored 1+, and the third scored 3+.

Sample 3: One pathologist scored 0+, the second scored 2+, and the third pathologist scored 2+.

Sample 4 and 5: One pathologist scored 0+, the second scored 2+, and the third scored 2+.

Sample 6: One pathologist scored 0+, the second scored 3+, and the third scored 3+.

Table 15. Cohort 2: PATHWAY HER-2/neu (CB11) Scoring for the Three Pathologists.

HER2 Score	PATHWAY HER-2/neu (CB11) Score		
	Investigator 4	Investigator 5	Investigator 6
3+	31	37	28
2+	38	32	47
0,1+	75	75	69
Total	144	144	144

Note: A total of 8 samples varied by more than one grade level (i.e., 0 - 2+) when evaluated by the three Pathologists.

Samples 1-6: One pathologist scored 0+, the second scored 1+, and the third scored 2+.

Samples 7 and 8: One pathologist scored 0+, the second scored 2+, and the third scored 2+.

Following is a tabulation of the ranges of percent agreements across pairs of pathologists (three pairs for each cohort).

Table 16. Ranges of 2X2* Agreements for the Three Pathologists.

	Overall Percent Agreement	Positive Percent Agreement	Negative Percent Agreement
Clone 4B5 vs. PATHWAY HER-2/neu (CB11)			
Cohort 1	82.6 – 86.9%	97.3 – 100.0%	68.0% - 75.4%
Cohort 2	88.2 – 95.5%	87.6 – 95.6%	86.1 – 95.4%
Clone 4B5 vs. FISH			
Cohort 1	86.8 – 88.2%	90.7 – 94.2%	79.3 – 81.0%
Cohort 2	87.4 – 89.9%	88.2 – 90.0%	84.5 – 91.8%
PATHWAY HER-2/neu (CB11) vs. FISH			
Cohort 1	79.9 – 84.0%	73.3 – 80.2%	89.7 – 89.7%
Cohort 2	84.8% - 93.3%	86.7 – 92.5%	82.7 – 94.1%

* 0, 1+ = Negative. 2+ and 3+ = Positive.

Conclusion: Data from these studies indicated that the clone 4B5 primary antibody was specific and reproducible in its ability to locate appropriate membrane staining for normal and neoplastic tissues. The method comparison data demonstrated that clone 4B5 primary antibody is indicated as an aid in the assessment of breast cancer patients for whom Herceptin treatment is considered.

9. Performance characteristics on BenchMark ULTRA instrument using iVIEW DAB Detection Kit or *ultra*View Universal DAB Detection Kit:

BenchMark ULTRA instrument inter-laboratory staining and inter-day reproducibility: Three laboratories, from separate institutions in the United States, participated in the inter-laboratory reproducibility study. Cut slides of 48 FFPE invasive breast carcinoma cases [12 each from each HER2 binning category (0, 1+, 2+, 3+)] and 1 pair of PATHWAY HER-2 4 in 1 Control Slides per each of 12 staining runs were distributed to study sites for staining on a VENTANA BenchMark ULTRA automated slide staining device using the recommended staining protocol and *ultra*View Universal DAB Detection Kit. Controls included the PATHWAY HER-2 4 in 1 Control Slides and a second slide of each case stained with negative Ig reagent. Pathologists, blinded to case status, evaluated the slides and provided a clinical score (i.e. 0, 1+, 2+, 3+). The results were analyzed by Ventana. Using standard nomenclature for 2x2 tables, average positive agreement (APA) across sites was calculated as [2a/(2a+b+c)] and average negative agreement (ANA) was calculated as [2d/(2d+b+c)]. Across all sites, the inter-site APA based on clinical assessment (positive, negative) was 90.0% (108/120) and the ANA was 92.9% (156/168). For pair-wise comparisons of sites, APA was calculated as a/(a+c) and ANA was calculated as d/(b+d). The inter-site APA rates were 93.0% (40/43), 87.2% (34/39), and 89.5% (34/38) for Site A vs. Site B, Site A vs. Site C, and Site B vs. Site C, respectively. The inter-site ANA rates were 94.3% (50/53), 91.2% (52/57), and 93.1% (54/58) for Site A vs. Site B, Site A vs. Site C, and Site B vs. Site C, respectively.

The following tables are 3x3 presentations of results for each reader based on clinical score where 2+ and 3+ were separated.

Table 17. Site A vs. Site B Inter-laboratory Agreement Rates 3x3 Analysis–clone 4B5 BenchMark ULTRA with *ultra*View Universal DAB Detection Kit.

Site A	Site B			
	3+	2+	0, 1+	Total
3+	12	2	0	14
2+	0	6	2	8
0, 1+	0	1	25	26
Total	12	9	27	48
Overall percent agreement (OPA): n/N (%)				43/48 (89.6)

Table 18. Site A vs. Site C Inter-laboratory Agreement Rates 3x3 Analysis–clone 4B5 BenchMark ULTRA instrument with *ultra*View Universal DAB Detection Kit.

	Site C			
Site A	3+	2+	0, 1+	Total
3+	12	1	1	14
2+	0	4	4	8
0, 1+	0	0	26	26
Total	12	5	31	48
Overall percent agreement (OPA): n/N (%)				42/48 (87.5)

Table 19. Site B vs. Site C Inter-laboratory Agreement Rates 3x3 Analysis–clone 4B5 BenchMark ULTRA instrument with *ultra*View Universal DAB Detection Kit.

	Site C			
Site B	**3+**	**2+**	**0, 1+**	**Total**
3+	12	0	0	12
2+	0	5	4	9
0, 1+	0	0	27	27
Total	12	5	31	48
Overall percent agreement (OPA): n/N (%)				44/48 (91.7)

10. BenchMark ULTRA instrument inter-day staining reproducibility:

    The inter-day reproducibility (IDR) portion of the study included 12 cases with an intended distribution of approximately three (3) cases at each clinical score (0, 1+, 2+, 3+). In total, the five runs on the BenchMark ULTRA instrument at the single institution (Site C) conducting the IDR portion of the study took place over a minimum of 20 days, such that no two staining days were consecutive. The IDR APA and ANA rates based on clinical assessment of clone 4B5 staining at Site C across all days were both 100%. The overall percent agreement rates (OPA) rates for inter-day comparisons based on clinical scores were 100% for each of the day-to-day comparisons and for all days combined.

11. Comparison study of BenchMark ULTRA staining platform to BenchMark XT staining platforms:

    Two staining laboratories and three reading sites in the United States participated in the platform comparison study. Cut slides of 280 FFPE invasive breast carcinoma cases [approximately 70 cases from each HER2 binning category (0, 1+, 2+, 3+)] were randomly distributed to two staining sites (140 cases to each site) for staining on a BenchMark XT instrument and a BenchMark ULTRA instrument using the respective recommended staining protocols and *ultra*View Universal DAB Detection Kit. Controls included the PATHWAY HER-2 4 in 1 Control Slides and a second slide of each case stained with negative Ig reagent. Stained cases from Site 1 and Site 2 were divided into four slide sets and provided, one set at a time, to three different qualified readers (pathologists), one reader at Site 1, one at Site 2, and one at Site 3. The pathologists, blinded to case status and staining platform, evaluated all four sets of slides and provided a clinical score (i.e., 0, 1+, 2+, 3+) for each case. The results were analyzed by Ventana. The PPA rates (and lower bound of the two-sided 95% confidence intervals) for clone 4B5 antibody staining on the BenchMark ULTRA instrument versus BenchMark XT instrument based on clinical assessment (positive, negative) were 91.6% (85.9), 91.2% (85.3), and 94.9% (89.3) for Reader A, B, and C, respectively. The NPA rates (and lower bound of the two-sided 95% confidence intervals) for clone 4B5 antibody staining on the BenchMark ULTRA instrument versus BenchMark XT instrument based on clinical assessment (positive, negative) were 91.9 (85.8), 93.8% (88.3), and 99.3 (96.3) for Reader A, B, and C, respectively. The OPA between the clone 4B5 staining using BenchMark ULTRA instrument versus BenchMark XT instrument based on 2x2 analysis of clinical assessment (positive, negative) was 91.8%, 92.5%, and 97.4% per Reader A, B, and C, respectively. The 3x3 presentation of inter-platform agreement rates for each reader based on clinical score (0/1+, 2+, 3+) are shown in the tables below:

Table 20. BenchMark ULTRA instrument vs. BenchMark XT instrument Inter-Platform Agreement Rates 3x3 Analysis–Reader A.

BenchMark ULTRA instrument	BenchMark XT instrument			
Reader A	3+	2+	0, 1+	Total
3+	84	11	1	96
2+	8	28	9	45
0, 1+	4	8	114	126
Total	96	47	124	267
Overall percent agreement: n/N (%) (95% CI)				226/267 (84.6) (79.8-88.5)

Table 21. BenchMark ULTRA instrument vs. BenchMark XT instrument Inter-Platform Agreement Rates 3x3 Analysis–Reader B.

BenchMark ULTRA instrument	BenchMark XT instrument			
Reader B	3+	2+	0, 1+	Total
3+	64	2	1	67
2+	3	56	7	66
0, 1+	2	10	122	134
Total	69	68	130	267
Overall percent agreement: n/N (%) (95% CI)				242/267 (90.6) (86.5-93.6)

Table 22. BenchMark ULTRA instrument vs. BenchMark XT instrument Inter-Platform Agreement Rates 3x3 Analysis–Reader C.

BenchMark ULTRA instrument	BenchMark XT instrument			
Reader C	3+	2+	0, 1+	Total
3+	64	1	0	65
2+	2	45	1	48
0, 1+	0	6	148	154
Total	66	52	149	267
Overall percent agreement: n/N (%) (95% CI)				257/267 (96.3) (93.2-98.0)

12. Inter-pathologist reproducibility of platform comparison study specimens:

    Positive and negative agreement rates with two-sided score 95% confidence intervals were calculated for the six possible pairwise comparisons between readers for each platform.

    For BenchMark ULTRA instrument, PPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs. A, and C vs. B were 94.7% (126/133), 98.2% (111/113), 98.2% (111/113), 89.4% (126/141), 78.7% (111/141), and 83.5% (111/133), respectively. NPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs, A, and C vs. B were 88.8% (119/134), 80.5% (124/154), 85.7% (132/154), 94.4% (119/126), 98.4% (124/126), and 98.5% (132/134), respectively. The OPA rate was highest between Reader A and Reader B (91.8%) and lower between Reader B and Reader C (91.0%) and Reader A and Reader C (88.8%).

    For BenchMark XT instrument, PPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs. A, and C vs. B were 94.9% (130/137), 98.3% (116/118), 98.3% (116/118), 90.9% (130/143), 81.1% (116/143), and 84.7% (116/137), respectively. NPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs. A, and C vs. B were 90.0% (117/130), 81.9% (122/149), 85.9% (128/149), 94.4% (117/124), 98.4% (122/124), and 98.5% (128/130), respectively. The OPA rate was highest between Reader A and Reader B (92.5%) and lower between Reader B and Reader C (91.4 %) and Reader A and Reader C (89.1%).

13. Comparison study of *i*VIEW DAB Detection Kit to ***ultra***View Universal DAB Detection Kit:

The Site 1 cohort of 140 FFPE invasive breast carcinoma cases [approximately 35 cases from each HER-2 binning category (0, 1+, 2+, 3+)] was used in a comparison study of *i*VIEW DAB Detection Kit to *ultra*View Universal DAB Detection Kit when staining with clone 4B5 on BenchMark ULTRA automated slide staining device. A single staining laboratory and three reading sites in the United States participated in the detection comparison study. For clone 4B5 antibody staining on the BenchMark ULTRA instrument the PPA rates between results obtained using *i*VIEW DAB Detection Kit and *ultra*View Universal DAB Detection Kit methods based on clinical assessment (positive, negative) were 95.8% (68/71), 96.9% (63/65), and 96.5% (55/57) for Readers A, B, and C, respectively and the NPA rates between detection methods were 90.8% (59/65), 91.5% (65/71), and 97.5% (77/79) for Readers A, B, and C, respectively. The OPA rates between detection kits were 93.4% (127/136), 94.1% (128/136), and 97.1% (132/136) for Readers A, B, and C, respectively. The 3x3 presentation of detection comparison agreement rates for each reader based on clinical score (0/1+, 2+, 3+) are shown in the tables below:

Table 23. Reader A, *i*VIEW DAB Detection Kit vs. *ultra*View Universal DAB Detection Kit Agreement Rates 3x3 Analysis–clone 4B5 Staining on BenchMark ULTRA instrument.

*i*VIEW DAB Detection Kit	***ultra***View Universal DAB Detection Kit			
Reader A	3+	2+	0, 1+	Total
3+	43	5	0	48
2+	3	17	6	26
0, 1+	0	3	59	62
Total	46	25	65	136
Overall percent agreement: n/N (%) (95% CI)			119/136 (87.5) (80.9-92.0)	

Table 24. Reader B, *i*VIEW DAB Detection Kit vs. *ultra*View Universal DAB Detection Kit Agreement Rates 3x3 Analysis–clone 4B5 Staining on BenchMark ULTRA instrument.

*i*VIEW DAB Detection Kit	***ultra***View Universal DAB Detection Kit			
Reader B	3+	2+	0, 1+	Total
3+	32	0	0	32
2+	0	31	6	37
0, 1+	1	1	65	67
Total	33	32	71	136
Overall percent agreement: n/N (%) (95% CI)			128/136 (94.1) (88.8-97.0)	

Table 25. Reader C, *i*VIEW DAB Detection Kit vs. *ultra*View Universal DAB Detection Kit Agreement Rates 3x3 Analysis–clone 4B5 Staining on BenchMark ULTRA instrument.

*i*VIEW DAB Detection Kit	***ultra***View Universal DAB Detection Kit			
Reader C	3+	2+	0, 1+	Total
3+	32	0	0	32
2+	0	23	2	25
0, 1+	0	2	77	79
Total	32	25	79	136
Overall percent agreement: n/N (%) (95% CI)			132/136 (97.1) (92.7-98.9)	

14. Inter-pathologist reproducibility of detection comparison study specimens:

Positive and negative agreement rates with two-sided score 95% confidence intervals were calculated for the six possible pairwise comparisons between readers for each method.

For *i*VIEW DAB Detection Kit, PPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs. A, and C vs. B were 100.0% (69/69), 98.2% (56/57), 96.5% (55/57), 93.2% (69/74), 75.7% (56/74), and 79.7% (55/69) respectively. NPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs, A, and C vs. B were 92.5% (62/67), 77.2% (61/79), 82.3% (65/79), 100.0% (62/62), 98.4% (61/62), and 97.0% (65/67) respectively. The overall agreement rate was highest between Reader A and Reader B (96.3%) and lower between Reader A and Reader C (86.0%) and Reader B and Reader C (88.2%).

For *ultra*View Universal DAB Detection Kit, PPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs. A, and C vs. B were 96.9% (63/65), 98.2% (56/57), 98.2% (56/57), 88.7% (63/71), 78.9% (56/71), and 86.2% (56/65), respectively. NPA rates for Reader A vs. B, A vs. C, B vs. C, B vs. A, C vs. A, and C vs. B were 88.7% (63/71), 81.0% (64/79), 88.6% (70/79), 96.9% (63/65), 98.5% (64/65), and 98.6% (70/71), respectively. The overall agreement rates were similar for each pair of readers, 92.6% (126/136), 88.2% (120/136), and 92.6% (126/136) for Reader A vs. B, Reader A vs. C, and Reader B vs. C, respectively.

PERFORMANCE CHARACTERISTICS IN GASTRIC CASES

1. Inter-run repeatability on the BenchMark XT instrument was performed in five runs conducted over a 5 day (non-consecutive) period. Five slides containing three gastric tissue cases with scores of 0, 1+, 2+, and 3+ HER2 expression demonstrated 100% agreement within the positive/negative value for each tissue.
2. Intra-run repeatability on the BenchMark XT instrument was performed on 28 slides containing three gastric tissue cases with scores of 0, 1+, 2+, and 3+ HER2 expression. All cases scored equivalently within the positive/negative value for each tissue type.

   Intra-platform repeatability was performed across three BenchMark XT instruments. In these runs all 30 slides from each of two different multi tissue blocks containing three gastric tissue cases with scores of 0, 1+, 2+, and 3+ HER2 expression scored equivalently within the positive/negative value for each tissue type.

   Intra-platform repeatability was tested across three BenchMark ULTRA instruments. In these runs all 15 slides from one multi-tissue block scored equivalently within the positive/negative value for each tissue type.

   Inter-platform repeatability was tested across three BenchMark XT and three BenchMark ULTRA instruments. In these runs all 30 slides from one multi-tissue block scored equivalently within the positive/negative value for each tissue type.
3. Comparison of *i*VIEW DAB Detection Kit and ***ultra***View Universal DAB Detection Kit using Gastric Cases:

   Clone 4B5 was used to conduct detection kit comparison testing across two instruments (BenchMark XT instrument and BenchMark ULTRA instrument), using *i*VIEW DAB Detection Kit and *ultra*View Universal DAB Detection Kit. Two hundred and ten tissue cases were used as part of the testing. The stained slides were evaluated for positive/negative clinical scoring.

   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 the following tables.

Table 26. Clinical assessment for *ultra*View Universal DAB Detection Kit versus *i*VIEW DAB Detection Kit on the BenchMark XT Instrument.

ultraView Universal DAB Detection Kit	*i*VIEW DAB Detection Kit		
	Positive	Negative	Total
Positive	21	0	21
Negative	0	189	189
Total	21	189	210
	n/N	%	
Positive percent agreement	21/21	100	
Negative percent agreement	189/189	100	
Overall percent agreement	210/210	100	

Table 27. Clinical assessment comparison on the BenchMark XT and BenchMark ULTRA staining platforms using *ultra*View Universal DAB Detection Kit.

BenchMark XT Staining Platform with ***ultra***View Universal DAB Detection Kit	BenchMark ULTRA Staining Platform with ***ultra***View Universal DAB Detection Kit		
	Positive	Negative	Total
Positive	20	1	21
Negative	0	189	189
Total	20	190	210
	n/N	% (95% CI)	
Positive percent agreement	20/20	100 (83.9-100)	
Negative percent agreement	189/190	99.5 (97.1-99.9)	
Overall percent agreement	209/210	99.5 (97.4-99.9)	

4. Comparison of Clone 4B5 to HercepTest in Human Gastric Cancer:

   A blinded, external study was conducted to compare the staining performance of the clone 4B5 on the BenchMark XT instrument to that of the Dako HercepTest. Approximately 239 cases of gastric cancer were tested for the study along with 159 cases from TARGOS laboratory, from the ToGA trial that investigated HER2 status and clinical outcome in patients treated with Herceptin. The laboratory stained the cases with clone 4B5 and HercepTest. A pathologist scored the cases on a scale of 0/1+, 2+, and 3+. Positive cases consist of scores of 2+ and 3+, while negative cases are 0 and 1+.

   Tables 28 and 29 include agreement rates between clone 4B5 and HercepTest, broken out by tissue source. Table 28 compares positive vs. negative outcomes, whereas table 29 uses a 3-category IHC scale of 0/1+, 2+, and 3+. The overall agreement for all tissues in tables 28 and 29 is 91.0% and 95.3% respectively.

Table 28. Percent Agreement Rates and 95% CI for clone 4B5 (IHC) vs. HercepTest broken out by tissue source. Both IHC tests were scored as positive vs. negative (0/1+ vs 2+/3+).

n	Overall Percent Agreement (95% CI)	n	Positive Percent Agreement (95% CI)	n	Negative Percent Agreement (95% CI)
362/398	91.0 (87.7-93.4)	46/56	82.1 (70.2-90.0)	316/342	92.4 (89.1-94.8)

Table 29. Overall Percent Agreement and 95% CI for clone 4B5 (IHC) vs. HercepTest Scores broken out by tissue source. IHC tests were scored as 0/1+, 2+, or 3+.

Tissue Source	n	Overall Percent Agreement (95% CI)
TMA & ToGA	355/398	89.2 (85.8-91.9)

5. Inter-laboratory reproducibility of clone 4B5: The study was conducted at three test sites. Specimens were selected for inclusion in the study based on clone 4B5 IHC clinical score, such that there were an approximately equal number of positive (3+) and negative (0, 1+) cases. Additionally, up to four cases of 2+ qualified gastric cancer cases were studied.

   The three sites each used a BenchMark XT platform and a BenchMark ULTRA platform to conduct four staining runs per platform. Cases were randomized for staining using a stratified randomization procedure that assigned cases such that each run contained cases representing all scoring categories for HER2 in gastric cancer. The runs on each instrument at each site contained the same cases. At each site, one slide from each case was stained with clone 4B5 and another slide from the same case was stained with CONFIRM Negative Control Rabbit Ig on the BenchMark ULTRA platform. A second pair of slides from the same case was similarly stained on the BenchMark XT platform at each site. Case slides were scored by one qualified reader at each site blinded to previously determined IHC clinical scores for each specimen.

   The overall agreement for all evaluable cases was 100% for all three site-to-site comparisons on both the BenchMark ULTRA platform and the BenchMark XT platform. The overall agreement between the BenchMark ULTRA platform and BenchMark XT platform for evaluable cases was 100% at each of the three sites. Background and morphology acceptability rates for all cases were 100% for both platforms at Sites A and C and >95% for both platforms at Site B. See tables below.

Table 30. Overall Clinical Assessment Agreement between Sites: All Evaluable Cases.

BenchMark ULTRA instrument	Percent Overall Agreement
Site A vs Site B: n/N (%) (95% CI)	30/30 (100%) (88.6 – 100)
Site A vs Site C: n/N (%) (95% CI)	30/30 (100%) (88.6 – 100)
Site B vs Site C: n/N (%) (95% CI)	30/30 (100%) (88.6 – 100)
BenchMark XT instrument	**Percent Overall Agreement**
Site A vs Site B: n/N (%) (95% CI)	31/31 (100%) (89.0 – 100.0)
Site A vs Site C: n/N (%) (95% CI)	31/31 (100%) (89.0 – 100.0)
Site B vs Site C: n/N (%) (95% CI)	31/31 (100%) (89.0 – 100.0)

Table 31. Overall Clinical Assessment Agreement between Platforms: All Evaluable Cases.

BenchMark ULTRA instrument vs BenchMark XT instrument	Percent Overall Agreement
Site A: n/N (%) (95% CI)	40/40 (100%) (91.2 – 100)
Site B: n/N (%) (95% CI)	34/34 (100%) (89.8 – 100)
Site C: n/N (%) (95% CI)	32/32 (100%) (89.3 – 100)

Table 32. Background Staining and Morphology Acceptability Rates: All Cases.

BenchMark ULTRA instrument	Site A	Site B	Site C
Morphology Acceptability Rates	44/44 (100%)	43/44 (97.7%)	44/44 (100%)
Background Acceptability Rates	44/44 (100%)	42/44 (95.5%)	44/44 (100%)
BenchMark XT instrument	**Site A**	**Site B**	**Site C**
Morphology Acceptability Rates	44/44 (100%)	43/44 (97.7%)	44/44 (100%)
Background Acceptability Rates	44/44 (100%)	43/44 (97.7%)	44/44 (100%)

6. Comparison study of BenchMark staining platform and BenchMark GX staining platform to BenchMark XT staining platform:

Cut slides of 3 TMAs containing FFPE gastric carcinoma cases [approximately 50 cases per TMA] were stained on a BenchMark XT instrument, BenchMark instrument and BenchMark GX instrument using the respective recommended staining protocols for *ultra*View Universal DAB Detection Kit and *i*VIEW DAB Detection Kit. Controls included the PATHWAY HER-2 4 in 1 Control Slides and a second slide of each TMA stained with negative Ig reagent. Stained slides were scored by one reader (pathologist).

The overall agreement rates (and lower bound of the two-sided 95% confidence intervals) for clone 4B5 antibody staining based on clinical assessment (positive, negative) were as follows: BenchMark instrument versus BenchMark XT instrument with *ultra*View Universal DAB Detection Kit 98.0% (94.2-99.3), BenchMark GX instrument versus BenchMark XT instrument with *ultra*View Universal DAB Detection Kit 97.4% (93.6-99.0), BenchMark instrument versus BenchMark XT instrument with *i*VIEW DAB Detection Kit 96.6% (92.7-98.4), BenchMark GX instrument versus BenchMark XT instrument with *i*VIEW DAB Detection Kit 95.9% (91.8-98.0).

The positive agreement rates (and lower bound of the two-sided 95% confidence intervals) for clone 4B5 antibody staining based on clinical assessment (positive, negative) were as follows: BenchMark instrument versus BenchMark XT instrument with *ultra*View Universal DAB Detection Kit 91.7% (64.4-98.5), BenchMark GX instrument versus BenchMark XT instrument with *ultra*View Universal DAB Detection Kit 78.6% (52.4-92.4), BenchMark instrument versus BenchMark XT with *i*VIEW DAB Detection Kit 80.0% (54.8-93.0), BenchMark GX instrument versus BenchMark XT with *i*VIEW DAB Detection Kit 73.3% (48.0-89.1).

The negative agreement rates (and lower bound of the two-sided 95% confidence intervals) for clone 4B5 staining based on clinical assessment (positive, negative) were as follows: BenchMark instrument versus BenchMark XT instrument with *ultra*View Universal DAB Detection Kit 98.5% (94.8-99.6), BenchMark GX instrument versus BenchMark XT instrument with *ultra*View Universal DAB Detection Kit 99.3% (96.1-99.9), BenchMark instrument versus BenchMark XT instrument with *i*VIEW DAB Detection Kit 98.1% (94.6-99.4), BenchMark GX instrument versus BenchMark XT instrument with *i*VIEW DAB Detection Kit 98.1% (94.5-99.3). The 2x2 presentation of the agreement rates for each comparison based on clinical assessment (positive, negative) are shown in the tables below.

Table 33. BenchMark instrument vs. BenchMark XT instrument Inter-Platform Agreement Rates with *ultra*View Universal DAB Detection Kit 2x2 Analysis.

Clone 4B5 with *ultra*View Universal DAB Detection Kit			
BenchMark instrument	BenchMark XT instrument		
	Positive	Negative	Total
Positive	11	2	13
Negative	1	133	134
Total	12	135	147
	n/N	% (95% CI)	
Overall percent agreement	144/147	98.0% (94.2-99.3)	
Positive percent agreement	11/12	91.7% (64.6-98.5)	
Negative percent agreement	133/135	98.5% (94.8-99.6)	

Table 34. BenchMark GX instrument vs. BenchMark XT instrument Inter-Platform Agreement Rates with *ultra*View Universal DAB Detection Kit 2x2 Analysis.

Clone 4B5 with *ultra*View Universal DAB Detection Kit			
BenchMark GX instrument	BenchMark XT instrument		
	Positive	Negative	Total
Positive	11	1	12
Negative	3	140	143
Total	14	141	155
	n/N	% (95% CI)	
Overall percent agreement	151/155	97.4% (93.6-99.0)	
Positive percent agreement	11/14	78.6% (52.4-92.4)	
Negative percent agreement	140/141	99.3% (96.1-99.9)	

Table 35. BenchMark instrument vs. BenchMark XT instrument Inter-Platform Agreement Rates with *i*VIEW DAB Detection Kit, 2x2 Analysis.

Clone 4B5 with *i*VIEW DAB Detection Kit			
BenchMark instrument	BenchMark XT instrument		
	Positive	Negative	Total
Positive	12	3	15
Negative	3	156	159
Total	15	159	174
	n/N	% (95% CI)	
Overall percent agreement	168/174	96.6% (92.7-98.4)	
Positive percent agreement	12/15	80.0% (54.8-93.0)	
Negative percent agreement	156/159	98.1% (94.6-99.4)	

Table 36. BenchMark GX instrument vs. BenchMark XT instrument Inter-Platform Agreement Rates with *i*VIEW DAB Detection Kit, 2x2 Analysis.

Clone 4B5 with *i*VIEW DAB Detection Kit			
BenchMark GX instrument	BenchMark XT instrument		
	Positive	Negative	Total
Positive	11	3	14
Negative	4	154	158
Total	15	157	172
	n/N	% (95% CI)	
Overall percent agreement	165/172	95.9% (91.8-98.0)	
Positive percent agreement	11/15	73.3% (48.0-89.1)	
Negative percent agreement	154/157	98.1% (94.5-99.3)	

Conclusion: Data from these studies indicate that the VENTANA HER2 (4B5) was specific and reproducible in its ability to appropriately stain breast and gastric neoplastic tissues. The method comparison and inter-laboratory reproducibility data demonstrated that VENTANA HER2 (4B5) is indicated as an aid in the assessment of breast and gastric cancer patients for whom Herceptin treatment is considered.

PERFORMANCE CHARACTERISTICS – PERJETA AND KADCYLA

HER2 Breast - Method Comparison Study to Enrollment Assay of Perjeta and Kadcyla studies in breast carcinoma

Equivalency to enrollment assays for cohorts from Perjeta and Kadcyla studies was determined by staining of trial specimens with VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody *assay*. 2753 specimens evaluated for a Perjeta trial and 99 specimens evaluated for a Kadcyla trial were stained with VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody (VENTANA HER2 (4B5)). *Positive (PPA), Negative (NPA) and Overall (OPA) Percent Agreement rates were determined.* The 95% CI (2-sided 95% confidence interval) was calculated using the score method.

Table 37. Agreement of the Clone 4B5 and Dako Assays on HER2 Status for all HER2 Evaluable Subjects. IHC Evaluable Subjects have a HER2 status of Positive or Negative determined by both the Clone 4B5 and the enrollment IHC assay.

			DAKO HER2 Status [a] [b]		
Study	Clone 4B5 Score [b]		Positive	Negative	*Total*
Perjeta and Kadcyla		3+	2380	15	2395
		2+	140	122	262
		0/1+	38	135	173
		Total	2558	272	2830
	Positive Percent Agreement (PPA) n/N (%) (95% CI)		2380/2558 (93.0) (92.0-94.0)		
	Negative Percent Agreement (NPA) n/N (%) (95% CI)		257/272 (94.5) (91.1-96.6)		
	Overall Percent Agreement (OPA) n/N (%) (95% CI)		2637/2830 (93.2) (92.2-94.1)		

[a] Positive = IHC Positive and/or ISH Amplified. Negative = IHC Negative and not ISH Amplified or ISH Non-Amplified and not IHC Positive.
[b] IHC: Positive = 3+; Negative = 0, 1+, or 2+.

Table 38. Agreement of Clone 4B5 and Dako Assays on IHC Status for all IHC Evaluable Subjects. IHC Evaluable Subjects have a HER2 status of Positive or Negative determined by both Clone 4B5 and the enrollment IHC assay.

		Dako HercepTest Status [a]		
Study	Clone 4B5 Status [a]	Positive	Negative	Total
Perjeta and Kadcyla	Positive	2330	65	2395
	Negative	21	414	435
	Total	2351	479	2830
	Positive Percent Agreement (PPA) n/N (%) (95% CI)	2330/2351 (99.1) (98.6-99.4)		
	Negative Percent Agreement (NPA) n/N (%) (95% CI)	414/479 (86.4) (83.1-89.2)		
	Overall Percent Agreement (OPA) n/N (%) (95% CI)	2744/2830 (97.0) (96.3-97.5)		
Perjeta	Positive	2267	63	2330
	Negative	10	399	409
	Total	2277	462	2739
	Positive Percent Agreement (PPA) n/N (%) (95% CI)	2267/2277 (99.6) (99.2-99.8)		
	Negative Percent Agreement (NPA) n/N (%) (95% CI)	399/462 (86.4) (82.9-89.2)		
	Overall Percent Agreement (OPA) n/N (%) (95% CI)	2666/2739 (97.3) (96.7-97.9)		
Kadcyla	Positive	63	2	65
	Negative	11	15	26
	Total	74	17	91
	Positive Percent Agreement (PPA) n/N (%) (95% CI)	63/74 (85.1) (75.3-91.5)		
	Negative Percent Agreement (NPA) n/N (%) (95% CI)	15/17 (88.2) (65.7-96.7)		
	Overall Percent Agreement (OPA) n/N (%) (95% CI)	78/91 (85.7) (77.1-91.5)		

[a] Positive = 3+; Negative = 0, 1+, or 2+.

Table 39. Agreement of Clone 4B5 and Dako Assays on IHC Score for all IHC Evaluable Subjects. IHC Evaluable Subjects have a HER2 status of Positive or Negative determined by both the Clone 4B5 and the enrollment IHC assay.

		Dako HercepTest Score			
Study	Clone 4B5 Score	3+	2+	0/1+	Total
Perjeta and Kadcyla	3+	2330	64	1	2395
	2+	12	235	15	262
	0/1+	9	26	138	173
	Total	2351	325	154	2830
	Overall Percent Agreement (OPA) n/N (%) (95% CI)	2703/2830 (95.5) (94.7-96.2)			
Perjeta	3+	2267	62	1	2330
	2+	9	226	13	248
	0/1+	1	24	136	161
	Total	2277	312	150	2739
	Overall Percent Agreement (OPA) n/N (%) (95% CI)	2629/2739 (96.0) (95.2-96.7)			
Kadcyla	3+	63	2	0	65
	2+	3	9	2	14
	0/1+	8	2	2	12
	Total	74	13	4	91
	Overall Percent Agreement (OPA) n/N (%) (95% CI)	74/91 (81.3) (72.1-88.0)			

Table 40. Clone 4B5 Staining Acceptability. IHC Tested Subjects. IHC staining is considered acceptable if a valid IHC score (0, 1+, 2+, or 3+) could be determined. Reasons for unacceptable staining include unacceptable negative control, tissue loss, insufficient tumor, unacceptable background, and unacceptable morphology.

Parameter	Perjeta	Kadcyla	Perjeta and Kadcyla
Number of Initial IHC Tests	2753	99	2852
Initial Staining Acceptability n/N (%) (95% CI)	2708/2753 (98.4) (97.8, 98.8)	92/99 (92.9) (86.1, 96.5)	2800/2852 (98.2) (97.6, 98.6)
Number of Repeat IHC Tests	40	0	40
Final Staining Acceptability n/N (%) (95% CI)	2746/2753 (99.7) (99.5, 99.9)	92/99 (92.9) (86.1, 96.5)	2838/2852 (99.5) (99.2, 99.7)

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same instrument run should be checked to determine if it is because of 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 bar code label. If the slide is labeled properly, other positive controls run on the same instrument run should be checked to determine if it is because of 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 all of the paraffin has not been removed, there may be no staining. The deparaffinization procedure should be repeated.
4. If specific antibody staining is too intense, the run should be repeated with incubation time shortened until the desired stain intensity is achieved.
5. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
6. If nuclear and cytoplasmic staining are present in normal mucosa in close proximity to the tumor area in gastric carcinoma, and confuses interpretation of membrane staining, the case can be tested by ISH.
7. For corrective action, refer to the Step By Step Procedure section, the instrument Operator's Manual or contact your local support representative.

REFERENCES

1. Akiyama T, et al. The product of the human c-erbB-2 Gene: A 185-kilodalton glycoprotein with tyrosine kinase activity. Science. 1986;232: 1644-1646.
2. Kraus MH, Popescu NC, Amsbaugh C, King RC. Overexpression of EGF receptor-related proto-oncogene *erb*B-2 in human mammary tumor cell lines by different molecular mechanisms. EMBO. 1987; 6: 605-610.
3. Dickson RB, and Lippman ME. Genes, Oncogenes, and Hormones. Boston: Kluwer Academic Publishers; 1992.
4. Keatings, L. et al. c-erbB-2 oncoprotein expression in mammary and extramammary Paget's disease: an immunohistochemical study. Histopathology. 1990;17: 234-247.
5. Herceptin (Trastuzamab) [Package Insert]. EMEA (European Medicines Agency). http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000278/WC500074922.pdf. Published 01/03/2010. Updated 04/02/2011. Accessed October 2010.
6. Roche PC. Immunohistochemical stains for breast cancer. Mayo Clin Proc. 1994;69: 57-58.
7. Charpin C, et al. c-erbB-2 oncoprotein detected by automated quantitative immunocytochemistry in breast carcinomas correlates with patients' overall and disease-free survival. Br J Cancer. 1997;75: 667-1673.
8. Corbett IP, et al. NCL-CB11: a new monoclonal antibody recognizing the internal domain of the c-erbB-2 oncogene protein, effective for use on formalin fixed, paraffin-embedded tissue. J Pathol. 1990;161:15-25.
9. Nicholson RI, et al. Relationship between EGF-R, c-erbB-2 protein expression and Ki67 immunostaining in breast cancer hormone sensitivity. Eur J Cancer. 1993;29A:1018-1023.
10. DePotter CR, et al. The expression of the *neu* oncogene product in breast lesions and in normal fetal and adult human tissues. Histopathology. 1989;15:351-362.
11. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
12. Department of Health, Education and Welfare, National Institute of Occupational Safety and Health, Rockville, MD. "Procedures for the decontamination of plumbing systems containing copper and/or lead azides." DHHS (NIOSH) Publ No. 78-127, Current 13. August 16, 1976.
13. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2010.
14. 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.
15. Hoffmann M, et al. Histopathology. 2008:52;797-805.
16. Rüschoff J, Dietel M, Baretton G, Arbogast S, Walch A, Monges G, Chenard MP, Penault-Llorca F, Nagelmeier I, Schlake W, Höfler H, Kreipe HH. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. Virchows Arch. 2010;457(3):299-307.
17. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66(4):194-199.
18. 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.
19. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med. 1983;14:767.
20. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.
21. Thomson TA, Hayes MM, Spinelli JJ, Hiland E, Sawrenko C, and Phillip D, et al. HER-2/neu in breast cancer: interobserver variability and performance of immunohistochemistry with 4 antibodies compared with fluorescent in situ hybridization, Mod Pathol. 2001;14:1079-86.
22. Kay EW, Walsh CJ, Cassidy M, Curran B, Leader M. C-erbB-2 immunostaining: problems with interpretation. J Clin Pathol. 1994;47:816-22.
23. Bilous M, Dowsett M, Hanna W, Isola J, Lebeau A, Moreno A, Penault-Llorca F, Rüschoff J, Tomasic G, van de Vijver M. Current Perspectives on HER2 Testing: A Review of National Testing Guidelines. Mod Pathol. 2003;16:173-182.
24. Rhodes A, Sarson J, et al. The reliability of rabbit monoclonal antibodies in the immunohistochemical assessment of estrogen receptors, progesterone receptors, and HER2 in human breast carcinomas. Am J Clin Pathol. 2010;134(4): 621-32.
25. van der Vegt B, de Bock GH, Bart J, Zwartjes NG, Wesseling J. Validation of the 4B5 rabbit monoclonal antibody in determining HER2 status in breast cancer. Mod Pathol. 2009;22(7):879-886.
26. Mayr D, Heim S, Werhan C, Zeindl-Eberhart E, Kirchner T. Comprehensive immunohistochemical analysis of Her-2/neu oncoprotein overexpression in breast cancer: HercepTest (Dako) for manual testing and Her-2/neu Test 4B5 (Ventana) for Ventana BenchMark automatic staining system with correlation to results of fluorescence in situ hybridization (FISH). Virchows Arch. 2009;454(3):241-8 (Epub: 24 Jan 2009).
27. Itoh H, Kato N, Serizawa A, Itoh T, Umemura S, Osamura RY. HER2 Rabbit Monoclonal Antibody 4B5 and Silver SISH: High Performance in Surgical Pathology for Appropriate Patient Care. Laboratory Investigation. 2009;89(1): 48A.
28. Powell WC, Roche PC, Tubbs RR. A New Rabbit Monoclonal Antibody (4B5) for the Immunohistochemical (IHC) Determination of the HER2 Status in Breast Cancer: Comparison With CB11, Fluorescence In Situ Hybridization (FISH), and Interlaboratory Reproducibility. Appl Immunohistochem Mol Morphol. 2008;16(6):569.

29. Carbone A, Botti G, et al. Delineation of HER2 gene status in breast carcinoma by silver in situ hybridization is reproducible among laboratories and pathologists. J Mol Diagn. 2008;10(6): 527-36.
30. Wolff AC, Hammond ME, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. Arch Pathol Lab Med. 2007;131(1):18.
31. Baselga J, Gelmon KA, Verma S, Wardley A, Conte P, Miles D, Bianchi G, Cortes J, McNally VA, Ross GA, Fumoleau P, Gianni L. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. J Clin Oncol. 2010 Mar 1;28(7):1138-44.
32. Swain SM, Kim SB, Cortés J, Ro J, Semiglazov V, Campone M, Ciruelos E, Ferrero JM, Schneeweiss A, Knott A, Clark E, Ross G, Benyunes MC, Baselga J. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase 3 study. Lancet Oncol. 2013 May;14(6):461-71.
33. Hurvitz SA, Dirix L, Kocsis J, Bianchi GV, Lu J, Vinholes J, Guardino E, Song C, Tong B, Ng V, Chu YW, Perez EA. Phase II randomized study of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. J Clin Oncol. 2013 Mar 20;31(9):1157-63.
34. http://www.kadcyla.com/hcp
35. http://www.perjeta.com/hcp

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany

www.ventana.com