# *ultra*View SISH DNP Detection Kit

REF 760-098

05572037001

IVD Σ 100

## INTENDED USE

Ventana Medical Systems, Inc.'s (Ventana) *ultra*View SISH DNP Detection Kit is an indirect, biotin-free system for detecting DNP labeled probes. The kit is intended to identify targets by silver in situ hybridization (ISH) in sections of formalin-fixed, paraffin-embedded tissue that are stained on VENTANA Benchmark XT automated slide stainer or VENTANA BenchMark ULTRA automated slide stainer instruments.

The clinical interpretation of any staining, or the absence of staining, must be complemented by histological examination and evaluation of proper controls. Evaluation must be made by a qualified reader within the context of the patient's clinical history and other diagnostic tests.

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

## SUMMARY AND EXPLANATION

In general, in situ hybridization (ISH) uses labeled probes to detect specific DNA or RNA target sequences in fixed tissue. This is accomplished by heating the tissue and probe solution to denature nucleic acids. The reaction is then cooled, allowing the labeled nucleic acid probe to hybridize to its endogenous complementary target sequence in the tissue.

The hybridization of the probe to the target sequence is visualized with an indirect detection method that locates the bound probe and generates a signal. The most common techniques for indirect methods use a secondary antibody directed against the species of primary antibody (anti-hapten) and an enzyme with a corresponding substrate chromogen system. This combination results in a colored precipitate at the site of specific antibody binding. The *ultra*View SISH DNP Detection Kit uses the indirect method to visualize specific antibodies bound to antigens by depositing a black colored precipitate.

## PRINCIPLE OF THE PROCEDURE

The *ultra*View SISH DNP Detection Kit detects dinitrophenyl (DNP) labeled probes bound to target sequences using silver in situ hybridization (SISH) in paraffin embedded tissue sections. First, the section is hybridized with a DNP-labeled probe, followed by incubation with a rabbit anti-DNP antibody, which binds to the DNP hapten on the probe. A multimer solution, a goat anti-rabbit secondary antibody with a horseradish peroxidase (HRP) enzyme, is applied to detect the rabbit anti-DNP antibody. The visualization of the bound secondary antibody is accomplished with enzyme (HRP) catalyzed deposition of silver which produces a black precipitate. Silver ions ($Ag^+$) from the Silver ISH DNP Chromogen A (Silver A) solution are reduced by hydroquinone from the Silver ISH DNP Chromogen B (Silver B) solution to metallic silver ions ($Ag^0$). This reaction is fueled by the substrate for HRP, hydrogen peroxide (Silver C). The silver precipitate is deposited in the nuclei and the target sequence is visualized as a black dot, which is readily visualized by light microscopy. Figure 1 illustrates the SISH reaction. The specimen is then counterstained with Hematoxylin II for interpretation by light microscopy.

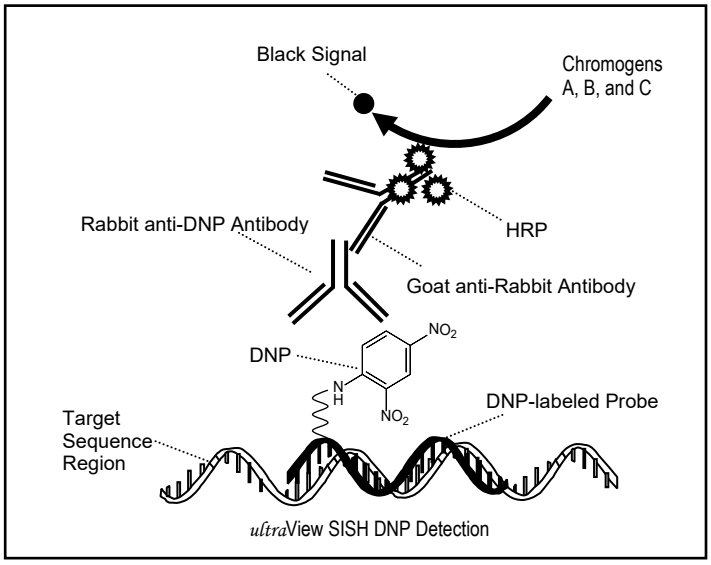


**Figure 1. SISH Reaction**

The *ultra*View SISH DNP Detection Kit is optimized for use with VENTANA probes, accessory reagents, and VENTANA BenchMark XT or BenchMark ULTRA automated slide stainers. The staining protocol consists of numerous steps in which reagents are incubated for precise times at specific temperatures. At the end of each incubation step, the VENTANA automated slide stainer washes the sections to remove unbound material and applies a liquid coverslip, which minimizes the evaporation of the aqueous reagents from the slide.[1] Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes.

For more detailed information on instrument operation, refer to the appropriate VENTANA automated slide stainer Operator's Manual.

## MATERIAL AND METHODS

### Material Provided

*ultra*View SISH DNP Detection Kit provides reagents sufficient for 100 tests.

| | |
|---|---|
| One 10 mL dispenser | *ultra*View SISH DNP Rabbit anti-DNP Antibody contains approximately 150 µg/mL of a rabbit monoclonal antibody directed against DNP diluted in a buffer containing carrier protein plus 0.05% ProClin 300, a preservative.* |
| One 10 mL dispenser | *ultra*View SISH DNP HRP contains a horseradish peroxidase (HRP) conjugated goat anti-rabbit antibody (approximately 20 µg/mL) in a protein stabilized buffer plus 0.05% ProClin 300, a preservative.* |
| One 20 mL dispenser | *ultra*View SISH DNP Chromogen A contains approximately 1% CH3COOAg in an aqueous solution. |
| One 10 mL dispenser | *ultra*View SISH DNP Chromogen B contains approximately 1% C6H6O2 in a citrate buffer solution. |
| One 10 mL dispenser | *ultra*View SISH DNP Chromogen C contains approximately 0.2% H2O2 in an aqueous solution. |

*For preservative information please refer to the Warnings and Precautions section.

### Reconstitution, Mixing, Dilution, Titration

The detection kit is optimized for use on Benchmark XT and BenchMark ULTRA instruments. No reconstitution, mixing, dilution, or titration of kit reagents is required. Further dilution may result in loss of staining.

## Materials Required but Not Provided

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

1. VENTANA DNP-labeled probe
2. Positive and negative tissue controls (consult probe method sheets for recommended types)
3. HybReady Solution (Cat. No. 780-4409 / 05917557001)
4. VENTANA Red ISH DIG Detection Kit (Cat. No. 760-512 / 08318832001)*
5. *ultra*View Silver Wash II (Cat. No. 780-003 / 05446724001)
6. ISH Block (Cat. No. 780-4461 / 05994918001)
7. Counterstain (as needed for specific applications)
8. ISH Protease 2 (Cat. No. 780-4148 / 05273323001)*
9. ISH Protease 3 (Cat. No. 780-4149 / 05273331001)*
10. Hematoxylin II (Cat. No. 780-2208 / 05277965001)*
11. Negative control reagent
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)*
13. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
14. SSC (10X) (Cat. No. 950-110 / 05353947001)
15. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
16. Cell Conditioning Solution (CC2) (Cat. No. 950-123 / 05279798001)*
17. ULTRA Cell Conditioning Solution (ULTRA CC2) (Cat. No. 950-223 / 05424542001)
18. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
19. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
20. BenchMark IHC/ISH instrument
21. Superfrost Plus microscope slides (VWR REF 48311-703 or equivalent)
22. Permanent mounting medium
23. Cover glass
24. General purpose laboratory equipment

* As needed for specific applications.

## Storage and Stability

Store at 2-8°C. Do not freeze. The user must validate any storage conditions other than those specified in the package insert. This detection kit can be used immediately after removal from the refrigerator.

To ensure proper reagent delivery and stability of each reagent, after every run replace dispenser cap and immediately place the dispenser in the refrigerator in an upright position.

Every detection kit is expiration dated. When properly stored, the reagents are stable to the date indicated on the label. Do not use detection kit beyond the expiration date. Your local support representative should be contacted immediately if there is an indication of reagent instability.

## Specimen Collection and Preparation for Analysis

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this reagent. Each section should be cut to the appropriate thickness (approximately 4 µm) and placed on a Superfrost Plus glass slide. The recommended tissue fixative is 10% neutral buffered formalin.[2] Ventana has determined that specimens fixed in zinc formalin or alcoholic formalin also are suitable specimen types. Specimens fixed in Prefer also exhibit single copy detection with this assay, yet the tissue morphology may be affected. It is not recommended that tissues fixed with AFA or Bouin's fixative be used with this assay. Specimens fixed >6 hours with AFA or Bouin's fixatives result in weak or absent staining, likely due to their acidic components hydrolyzing the nucleic acids in the samples.[3] Aside from the VENTANA assays, recent studies have found that the majority of inconclusive HER2 gene results by FISH relate to pre-analytic factors including under- and over-fixation,[4] as well as delayed fixation.[5] Strict implementation of fixation procedures (e.g., a dedicated processor to ensure a minimum of 6 hours fixation) resulted in a 64% reduction in inconclusive cases from 10.8% failures to 3.4%. Specimens fixed <6 hours in formalin can result in signal loss and nuclear over-digestion, as observed by pale/weak hematoxylin staining.

Sections thicker than 4 µm may require stronger protease treatment than the nominal condition and may exhibit more nuclear bubbling than thinner sections due to excess paraffin in the tissue. These may need to be deparaffinized in alcohol and xylene baths prior to staining on the instrument. Nuclear bubbling also may occur in two other contexts: 1) over-fixation (e.g., >24 hours) which may be remedied by the above mentioned deparaffinization procedure off-line, and 2) underfixation (1-3 hours with formalin) which is a less discrete nuclear bubbling. This may be remedied at 3 hours with changed cell conditioning/protease treatment, but at 1 hour is probably beyond remedy.

## WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. ProClin 300 solution is used as a preservative in this solution. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
6. Materials of human or animal origin should be handled as potentially biohazardous and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[10,11]
7. Take reasonable precautions when handling reagents. Avoid contact of reagents with eyes, skin, and mucous membranes. Use disposable gloves and wear suitable protective clothing when handling suspected carcinogens or toxic materials.
8. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
9. Ensure that the waste container is empty prior to starting a run on the instrument. If this precaution is not taken, the waste container may overflow and the user risks a slip and fall.
10. Avoid microbial contamination of reagents as this may produce incorrect results.
11. For further information on the use of this device, refer to the BenchMark XT and BenchMark ULTRA instrument User Guide, and method sheets of all necessary components located at navifyportal.roche.com.
12. Consult local and/or state authorities to determine the recommended method of disposal.
13. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
14. 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.
15. Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change.

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. |
| | H319 | Causes serious eye irritation |
| | P261 | Avoid breathing mist or vapours. |
| | P280 | Wear protective gloves/ eye protection/ face protection. |
| | P333 + P313 | If skin irritation or rash occurs: Get medical advice/ attention. |
| | P337 + P313 | If eye irritation persists: 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. |

EUH208 Contains Hydroquinone. May produce an allergic reaction.

## PROCEDURE

The *ultra*View SISH DNP Detection Kit has been developed for use on BenchMark XT and BenchMark ULTRA instruments in combination with VENTANA ancillary reagents. The staining protocols can be displayed, printed and edited according to the procedure in the instrument User Guide. Other operating parameters for the instrument have been preset at the factory.

The procedures for staining on BenchMark XT and BenchMark ULTRA instruments are as follows. For more detailed instructions and additional protocol options refer to the appropriate probe method sheet or your User Guide.

### BenchMark IHC/ISH Instruments

1. Apply slide bar code label which corresponds to the protocol to be performed.
2. Load the probe, appropriate detection kit dispensers, and required accessory reagent onto the reagent tray and place them on the instrument.
3. Check bulk fluids and empty waste.
4. Load the slides onto the instrument.
5. Start the staining run.
6. At the completion of the run, remove the slides from the instrument.
7. Proceed to Recommended Post-Instrument Processing Procedures.

### Recommended Post-Instrument Processing Procedures

#### Single staining assay with *ultra*View SISH DNP Detection Kit

Note: To ensure complete dehydration, ethanol baths need to be changed frequently and a third 100% ethanol bath may be added.

1. To remove Liquid Coverslip solution, wash the slides in 2 sequential solutions of a mild dishwashing detergent (do not use detergent designed for automatic dishwashers).
2. Rinse slides well with distilled water, about 1 minute. Shake off excess water.
3. Transfer the slides to an 80% ethanol bath for approximately 1 minute.
4. Transfer the slides to a 90% ethanol bath for approximately 1 minute.
5. Transfer the slides to a 100% ethanol bath for approximately 1 minute.
6. Transfer the slides into a second bath of 100% ethanol for approximately 1 minute.
7. Dip slides 10 times into 100% acetone (one time use only, replace acetone after each staining run). Do not leave slides in acetone.
8. Transfer the slides into the first xylene bath for approximately 30 seconds.
9. Transfer the slides into a second xylene bath for approximately 30 seconds.
10. Place coverslip on slide.

#### Dual staining assay in conjunction with *ultra*View Red ISH DIG Detection Kit

Note: Prolonged exposure to solvents, such as alcohol, acetone, and xylene, may result in decreased staining intensity when using the Fast Red chromogen. The recommended procedure is:

1. Wash the slides in a mild dishwashing detergent to remove the coverslip solution.
2. Rinse slides in deionized water for approximately 1 minute. Shake off excess water.
3. Dry slides for a minimum of 15 minutes in 60°C ± 5°C oven to avoid loss of signal due to moisture.
4. Apply coverslip to slides.
5. Failure to follow the recommended post instrument processing procedure may result in loss or unintended alteration of signal.

## QUALITY CONTROL PROCEDURE

### Positive Tissue Control

If applicable, see appropriate probe package insert.

### Negative Tissue Control

If applicable, see appropriate probe package insert.

### Positive Reagent Control

If applicable, see appropriate probe package insert.

### 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. Identify and correct the problem, then repeat the patient samples.

### Assay Verification

Prior to initial use of a probe or staining system in a diagnostic procedure, the specificity of the probe should be verified by testing it on a series of tissues with known ISH performance characteristics (refer to the probe method sheet) and to the Quality Control recommendations of CLSI Approved Guideline[6]. These quality control procedures should be repeated for each new lot or reagent, or whenever there is a change in assay parameters.

### Interpretation of Results

The VENTANA *ultra*View SISH DNP Detection Kit causes a black colored reaction product to precipitate at the sites localized by the probe and primary antibody. A qualified reader experienced in in situ hybridization procedures must evaluate controls and qualify the stained product before interpreting results. See detailed descriptions of tissue control evaluation in the appropriate probe package insert.

## LIMITATIONS

### General Limitations

1. ISH is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents and tissues, fixation, processing, preparation of the in situ hybridization 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 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 probes, 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 reagents are provided 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. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
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.7 Contact your local support representative with documented unexpected reactions.
8. False positive results may be seen because of non-immunological binding of proteins or substrate reaction products.

### Specific Limitations

1. Each step of the detection kit procedure has been optimized on the BenchMark XT and BenchMark ULTRA instruments and is preset. Because of variation in tissue fixation and processing, it may be necessary to modify some variables for individual specimens. . For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances"[8] or "Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist."[9]
2. The detection kit, in combination with VENTANA probes, primary antibodies and accessories, detects specific nucleic acid sequences that survive routine formalin, tissue processing and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.
3. As with any test, a negative result means that the specific nucleic acid sequence was not detected, not that the specific nucleic acid sequence was absent in the cells or tissue assayed.

4. This detection kit has been optimized for use with Reaction Buffer wash solution, primary antibodies, accessories, and BenchMark XT and BenchMark ULTRA instruments. The use of Reaction Buffer wash solution is important to the proper function of the detection kit. Users who deviate from recommended test procedures are responsible for interpretation of patient results under these circumstances.
5. This detection kit has been optimized for use with LCS (Predilute) or ULTRA LCS (Predilute). LCS is a prediluted coverslip solution used both as a barrier between aqueous reagents and the air as well as a reagent to remove paraffin from tissue samples during the deparaffinization process. The LCS barrier reduces evaporation and provides a stable aqueous environment for the IHC or in situ hybridization (ISH) reactions carried out on BenchMark XT and BenchMark ULTRA instruments.
6. All detection kits might not be registered on every instrument. Please contact your local support representative for more information.

## PERFORMANCE CHARACTERISTICS

### Pre-Clinical Studies

Lot to lot reproducibility of *ultra*View Red ISH DIG Detection Kit was determined by testing each of 3 lots of the INFORM HER2 Dual ISH DNA Probe Cocktail with 3 lots of *ultra*View SISH DNP and *ultra*VIew Red ISH DIG detection kits on duplicate slides of 3 human breast carcinoma cases and HER2 Dual ISH 3-in-1 Xenograft Slides. All slides (100%) passed slide adequacy and were enumerated by one qualified reader for raw HER2 and Chr 17 copies in 20 nuclei/specimen. The data were subjected to a variance component analysis based on a random effects model, and the results show that all acceptance criteria were met in this study. The %CVs across probe lot, detection kit lot, and within run all were <11%, indicating excellent precision of the assay.

### Clinical Studies

**Method Comparison Study: INFORM HER2 Dual ISH on BenchMark XT Instrument vs. PathVysion HER-2 FISH Kit**

The INFORM HER2 Dual ISH DNA Probe Cocktail assay utilizes the VENTANA *ultra*View Red ISH DIG Detection Kit for detection of Chromosome 17. To evaluate the clinical sensitivity and specificity of the INFORM HER2 Dual ISH DNA Probe Cocktail assay in determination of HER2 gene status, a multi-site method comparison study was performed using the Abbott/Vysis PathVysion HER-2 FISH Kit as the comparator device. Three clinical sites participated in the study. The clinical sites each provided ~200 cases of human breast cancer for potential inclusion in the study based on HER2/neu protein expression obtained previously with IHC. One central laboratory performed IHC staining on all samples using PATHWAY HER2/neu (4B5) assay for binning the samples. The clinical sites conducting the INFORM HER2 Dual ISH assay and the central laboratory conducting the PathVysion HER-2 FISH assay were blinded to IHC status and original case identifier to prevent bias in evaluation of the specimens. The FISH and INFORM HER2 Dual ISH DNA Probe Cocktail assay staining results were enumerated by counting at least 20 nuclei in each specimen. The results were reported as: HER2/Chr 17 ratio ≥2.0 as amplified; HER2/Chr 17 <2.0 as non-amplified. Of the 519 cases that were stained by both the FISH and INFORM HER2 Dual ISH assays, 510 specimens were enumerable by both assays and therefore included in the analysis of agreement rates.

The primary analysis compared positive and negative percent agreement rates to demonstrate equivalent performance between INFORM HER2 Dual ISH and PathVysion HER-2 FISH assays. Data for amplified and non-amplified clinical assessments for each assay, pooling data across all sites, are presented in a 2x2 table below along with percent positive and percent negative agreement rates where PathVysion HER-2 FISH is the reference assay. Acceptance criteria for demonstrating equivalent performance of these two assay methods when using the Benchmark XT platform required both positive and negative within-reader agreement rates of 90% or higher and two-sided 95% score confidence interval lower bounds of 85% or higher when pooling data from all three sites. These acceptance criteria were met (Table 2). Additionally, positive and negative agreement rates by site were all greater than 90% (Table 3).

For secondary efficacy results as well as inter-laboratory reproducibility see package insert of INFORM HER2 Dual ISH DNA Probe Cocktail [REF 780-4422].

**Table 2.** Amplification Status and Positive and Negative Agreement Rates for INFORM HER2 Dual ISH DNA Probe Cocktail and PathVysion HER-2 FISH Kit.

| | PathVysion HER-2 FISH Result | | |
|---|---|---|---|
| INFORM HER2 Dual ISH Result | Amplified | Non-Amplified | Total |
| Amplified | 216 | 22 | 238 |
| Non-Amplified | 9 | 263 | 272 |
| Total | 225 | 285 | 510 |
| | | | |
| | n/N | % (95% Score CI) | |
| Percent Positive Agreement | 216/225 | 96.0 (92.6-97.9) | |
| Percent Negative Agreement | 263/285 | 92.3 (88.6-94.8) | |

**Table 3.** Percent positive, percent negative and overall agreement rates between INFORM HER2 Dual ISH and PathVysion HER-2 FISH Kit, and 95% score confidence intervals, are presented by site.

| INFORM HER2 Dual ISH vs PathVysion HER-2 FISH | Percent Positive Agreement | Percent Negative Agreement | Percent Overall Agreement |
|---|---|---|---|
| Site A: n/N (%) (95% CI) | 75/79 (94.9) (87.7 – 98.0) | 85/92 (92.4) (85.1 – 96.3) | 160/171 (93.6) (88.8-96.4) |
| Site B: n/N (%) (95% CI) | 72/73 (98.6) (92.6-99.8) | 90/97 (92.8) (85.8-96.5) | 162/170 (95.3) (91.0-97.6) |
| Site C: n/N (%) (95% CI) | 69/73 (94.5) (86.7-97.8) | 88/96 (91.7) (84.4-95.7) | 157/169 (92.9) (88.0-95.9) |

These data indicate excellent agreement between the INFORM HER2 Dual ISH DNA Probe Cocktail assay and PathVysion HER-2 FISH Kit in determining HER2 gene status in human breast carcinoma specimens.

**Method Comparison Study: INFORM HER2 Dual ISH on BenchMark ULTRA Instrument vs. PathVysion HER2 FISH Kit**

To evaluate the clinical sensitivity and specificity of the INFORM HER2 Dual ISH DNA Probe Cocktail assay on BenchMark ULTRA instrument in determination of HER2 gene status, a multi-site method comparison study was performed using the Abbott/Vysis PathVysion HER-2 FISH Kit as the comparator device. The same three clinical sites participated in both the BenchMark XT and BenchMark ULTRA studies. Approximately ~500 cases of human breast cancer were included in the study based on HER2/neu protein expression obtained previously with IHC. These same cases were used for the method comparison study on the BenchMark XT instrument. Results of IHC staining using PATHWAY HER2/neu (4B5) assay and results of the PathVysion HER-2 FISH assay conducted at a central laboratory were obtained in the previous study. The clinical sites conducting the INFORM HER2 Dual ISH assay on BenchMark ULTRA instrument were blinded to original case identifier, IHC status, FISH status, and INFORM HER2 Dual ISH on BenchMark XT status to prevent bias in evaluation of the specimens. The FISH and INFORM HER2 Dual ISH DNA Probe Cocktail assay staining results were enumerated by counting at least 20 nuclei in each specimen. The results were reported as: HER2/Chr 17 ratio ≥2.0 as amplified; HER2/Chr 17 <2.0 as non-amplified. Of the 519 cases that were stained by both the FISH and INFORM HER2 Dual ISH on BenchMark ULTRA assays, 501 specimens were enumerable by both assays and therefore included in the analysis of agreement rates.

The primary analysis compared positive and negative percent agreement rates to demonstrate equivalent performance between INFORM HER2 Dual ISH and PathVysion HER-2 FISH assays. Data for amplified and non-amplified clinical assessments for each assay, pooling data across all sites, are presented in a 2x2 table below along with percent

positive and percent negative agreement rates where PathVysion HER-2 FISH is the reference assay. Acceptance criteria for demonstrating equivalent performance of these two assay methods when using the Benchmark ULTRA platform required both positive and negative within-reader agreement rates of 90% or higher and two-sided 95% score confidence interval lower bounds of 85% or higher when pooling data from all three sites. These acceptance criteria were met (Table 4). Additionally, positive and negative agreement rates by site were all greater than 90% (Table 5).

**Table 4.** Amplification Status and Positive and Negative Agreement Rates for INFORM HER2 Dual ISH DNA Probe Cocktail and PathVysion HER-2 FISH Kit.

| | PathVysion HER-2 FISH Result | | |
|---|---|---|---|
| INFORM HER2 Dual ISH Result | Amplified | Non-Amplified | Total |
| Amplified | 208 | 28 | 236 |
| Non-Amplified | 12 | 253 | 265 |
| Total | 220 | 281 | 501 |
| | | | |
| | n/N | % (95% Score CI) | |
| Percent Positive Agreement | 208/220 | 94.5 (90.7-96.9) | |
| Percent Negative Agreement | 253/281 | 90.0 (86.0-93.0) | |

**Table 5.** Percent positive, percent negative and overall agreement rates between INFORM HER2 Dual ISH and PathVysion HER-2 FISH Kit, and 95% score confidence intervals, are presented by site.

| INFORM HER2 Dual ISH vs PathVysion HER-2 FISH | Percent Positive Agreement | Percent Negative Agreement | Percent Overall Agreement |
|---|---|---|---|
| Site A: n/N (%) (95% CI) | 73/78 (93.6) (85.9-97.2) | 85/91 (93.4) (86.4-96.9) | 158/169 (93.5) (88.7-96.3) |
| Site B: n/N (%) (95% CI) | 67/68 (98.5) (92.1-99.7) | 82/93 (88.2) (80.1-93.3) | 149/161 (92.5) (87.4-95.7) |
| Site C: n/N (%) (95% CI) | 68/74 (91.9) (83.4-96.2) | 86/97 (88.7) (80.8-93.5) | 154/171 (90.1) (84.7-93.7) |

These data indicate excellent agreement between the INFORM HER2 Dual ISH DNA Probe Cocktail assay and PathVysion HER-2 FISH Kit in determining HER2 gene status in human breast carcinoma specimens.

For secondary efficacy results as well as inter-laboratory reproducibility see package insert of INFORM HER2 Dual ISH DNA Probe Cocktail [REF 780-4422].

## TROUBLESHOOTING

1. Refer to the Troubleshooting section of the appropriate probe method sheet.
2. Incomplete paraffin removal could result in staining artifacts or no staining.
   - If all paraffin has not been removed from the slide, the staining run should be repeated using the extended deparaffinization option, if available.
   - Alternatively, a manual off-instrument deparaffinization can be performed. If the manual option is used, deselect online deparaffinization from the staining protocol prior to loading the slides on the instrument. Extra care should be taken to ensure slides do not dry out prior to the staining run.
3. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged. Refer to the Specimen Collection and Preparation for Analysis section.
4. If sections thicker than 4 µm exhibit nuclear bubbling due to excess paraffin, select the “extended deparaffinization” option in the staining procedure.
5. For corrective action, refer to the Procedure section of the instrument User Guide, or contact your local support representative.
6. If a reagent dispenser does not dispense fluid, check the priming chamber or meniscus for foreign materials or particulates, such as fibers or precipitates. If the dispenser is blocked, do not use the dispenser and contact your local support representative. Otherwise, re-prime the dispenser by aiming the dispenser over a waste container, removing the nozzle cap, and pressing down on the top of the dispenser. Refer to the associated inline dispenser method sheet associated with P/N 800-098 for information about proper use.

## REFERENCES


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

### 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 |
| UDI | Unique Device Identifier |

## REVISION HISTORY

| Rev | Updates |
|---|---|
| D | Updates to Material and Methods, Warnings and Precautions, Procedure, Limitations, Troubleshooting, References and Symbols sections. |

## INTELLECTUAL PROPERTY

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