VENTANA ISH *i*VIEW Blue Plus Detection Kit

REF 760-097

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IVD Σ 100

INTENDED USE

Ventana Medical Systems, Inc.'s (Ventana) ISH *i*VIEW Blue Plus Detection Kit consists of a primary rabbit anti-DNP antibody followed by an indirect biotin streptavidin system for detecting VENTANA DNP labeled probes and antibodies. This kit is intended for staining sections of routinely fixed (neutral buffered formalin), paraffin embedded tissue on the BenchMark series automated slide stainers.

Light microscopy is used to detect the staining intensity. Positive results aid in the classification of normal and abnormal cells and tissues and serve as an adjunct to conventional histopathology tests.

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

This product 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 or frozen tissue sections. Target sequences are exposed 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 complementary nucleic acid sequence in the tissue.

The hybridization of the probe to the nucleic acid sequence is visualized with an indirect detection method. The most common indirect techniques use an antibody directed against an enzyme linked with a corresponding substrate-chromogen system. This combination results in a colored precipitate at the site of specific antibody binding. The *i*VIEW Blue Plus Detection Kit uses the indirect method to visualize complementary nucleic acid sequences by depositing a blue colored precipitate.

Immunohistochemistry (IHC) is a technique used in laboratories for diagnostic purposes. IHC uses specific primary antibodies to localize and bind to antigens in fixed or frozen tissue sections or cytology specimens. The binding of the antibody to the antigen is visualized with an indirect detection method. The most common techniques for indirect methods use a secondary antibody directed against the species of primary antibody and an enzyme with a corresponding substrate-chromogen system. This combination results in a colored precipitate at the site of specific antibody binding. ISH *i*VIEW Blue Plus Detection Kit uses an indirect method to visualize specific antibodies bound to antigens by depositing a blue colored precipitate.

PRINCIPLE OF THE PROCEDURE

ISH *i*VIEW Blue Plus Detection Kit detects specific DNP labeled probes and antibodies bound to a target sequence or antigen in paraffin-embedded tissue sections. The labeled probe or antibody is located by an anti-DNP antibody, then by an enzyme-labeled secondary antibody or a biotin-conjugated secondary antibody. This step is followed by the addition of a Streptavidin-AP (alkaline phosphatase) enzyme conjugate which binds to the biotin present on the secondary antibody. The complex is then visualized with 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) chromogen, which produces a blue precipitate that is readily detected by light microscopy.

The staining protocol consists of numerous steps in which reagents are incubated for pre-determined times at specific temperatures. At the end of each incubation step, the BenchMark IHC/ISH instrument washes the sections to remove unbound material and applies a liquid coverslip, which minimizes the evaporation of the aqueous reagents from the slide. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with positive staining for the probe or antibody.

For more detailed information on instrument operation, refer to the instrument's User Guide. Figure 1 illustrates the indirect detection method.

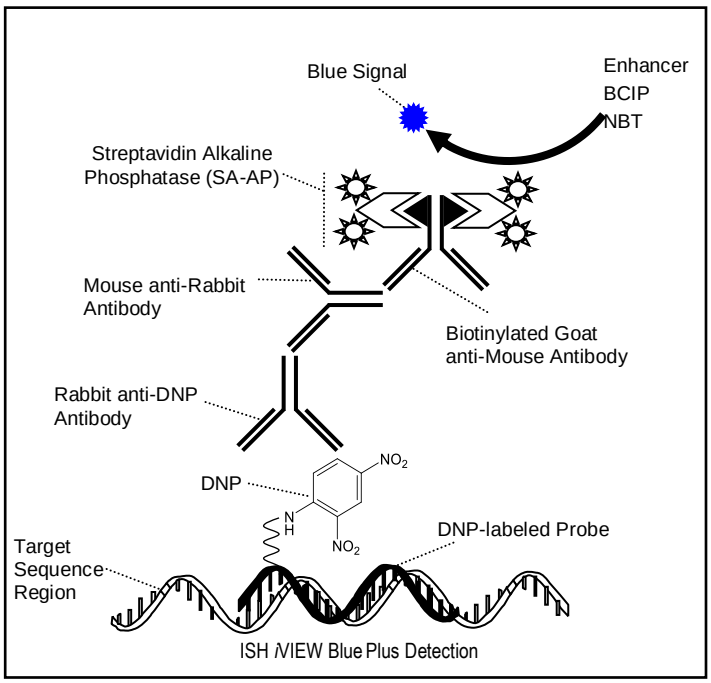

Figure 1. ISH *i*VIEW Blue Plus Reaction.

MATERIAL AND METHODS

Material Provided

ISH *i*VIEW Blue Plus Detection Kit contains sufficient reagent for 100 tests.

One 10 mL dispenser	ISH *i*VIEW Blue Plus Anti-DNP contains rabbit anti-DNP antibody in phosphate buffered saline with protein stabilizer and ProClin 300, a preservative (~ 1%).
One 10 mL dispenser	ISH *i*VIEW Blue Plus Streptavidin Alkaline Phosphatase contains enzyme conjugate in Tris buffer with $MgCl_2$ and $ZnCl_2$ (~ 1%).
One 10 mL dispenser	ISH *i*VIEW Blue Plus NBT contains nitro blue tetrazolium in ~ 1% dimethylformamide (DMF).
One 10 mL dispenser	ISH *i*VIEW Blue Plus BCIP contains 5-bromo-4-chloro-3-indolyl phosphate in a Tris buffer (~ 1%).
One 10 mL dispenser	ISH *i*VIEW Blue Plus Enhancer contains an $MgCl_2$ solution with ProClin 300, a preservative (~ 11%).
One 10 mL dispenser	ISH *i*VIEW Blue Plus Amp contains mouse anti-rabbit antibody in phosphate buffered saline with protein stabilizer and ProClin 300, a preservative (~ 1%).
One 10 mL dispenser	ISH *i*VIEW Blue Plus Biotinylated Ig contains affinity purified goat anti-mouse IgG in phosphate buffered saline with protein stabilizer and ProClin 300, a preservative (~ 1%).
One 10 mL dispenser	ISH *i*VIEW Blue Plus HybReady contains prehybridization buffer with formamide (~ 57% CH_3NO).

Reconstitution, Mixing, Dilution, Titration

The detection kit is optimized for use on BenchMark IHC/ISH instruments. No reconstitution, mixing, dilution, or titration of kit reagents is required.

Further dilution may result in loss of staining. The user must validate any such changes. Differences in tissue processing and pre-analytical conditions and procedures in the

laboratory may produce significant variability in results and require regular use of controls. For more information about controls, see the Quality Control Procedures section.

Materials Required but Not Provided

Staining reagents, such as VENTANA primary antibodies / probes and ancillary components, including negative and positive tissue control slides, are not provided.

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

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

1. DNP labeled ISH probe
2. DNP labeled antibody
3. Positive and negative tissue controls (consult probe method sheets for recommended types)
4. ISH Protease 1 (Cat. No. 780-4147 / 05273315001)*
5. ISH Protease 2 (Cat. No. 780-4148 / 05273323001)*
6. ISH Protease 3 (Cat. No. 780-4149 / 05273331001)*
7. Hematoxylin II Counterstain*
8. Bluing Reagent*
9. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
10. Negative reagent controls
11. ISH Block (Cat. No. 780-4461 / 05994918001)
12. SSC (10X) (Cat. No. 950-110 / 05353947001)
13. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
14. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
15. Cell Conditioning Solution (CC2) (Cat. No. 950-123 / 05279798001)
16. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
17. ULTRA Cell Conditioning Solution (ULTRA CC2) (Cat. No. 950-223 / 05424542001)
18. BenchMark IHC/ISH instrument
19. Slide barcode labels
20. Microscope slides, positively charged
21. Xylene (Histological grade)
22. Ethanol or reagent alcohol (Histological grade)
23. Deionized or distilled water
24. Permanent mounting medium (such as CytoSeal 60)
25. Coverslip sufficient to cover tissue
26. Automated coverslipper (such as Tissue-Tek SCA automated coverslipper)
27. Staining jars or baths
28. Oven capable of maintaining 60°C
29. Timer
30. Light microscope (20-80X)
31. Mild dishwashing detergent
32. General purpose laboratory equipment

* As needed for specific applications

Storage and Stability

Upon receipt and when not in use, store at 2-8°C. Do not freeze. 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 the 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 product beyond the expiration date. There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Your local support representative should be contacted immediately if unexpected results are observed.

Specimen Collection and Preparation for Analysis

Formalin-fixed, paraffin-embedded tissues are suitable for use with ISH *i*VIEW Blue Plus Detection Kit and a BenchMark IHC/ISH instrument (see Materials and Reagents Needed but Not Provided). The recommended tissue fixative is 10% neutral buffered formalin.[1] Variable results may occur as a result of tissue section thickness, fixation type, incomplete or prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness (2-5 µm) for the probe being used and placed on a glass microscope slide. Slides should be drained or dried to remove excess water between slide and tissue. Slide heating may be used to further enhance tissue adhesion to the glass. Consult the probe or antibody method sheet to identify heating limitations.

Sections thicker than 4 µm may require stronger protease treatment than the recommended condition and may exhibit more nuclear bubbling than thinner sections due to excess paraffin in the tissue. Nuclear bubbling appears as large or small bubbles or vacuoles in the nuclei. Usually this artifact does not push the SISH and Red ISH signals to the periphery of the nuclei or otherwise distort them, and therefore it does not interfere with signal enumeration. However, severe cases of nuclear bubbling may distort the nuclei or SISH and Red ISH and Blue ISH signals such that enumeration is not possible. These specimens may need to be deparaffinized in xylene and alcohol baths prior to repeat staining on the instrument, or the user may select the extended deparaffinization option in the staining procedure (see Troubleshooting). Nuclear bubbling also may occur in the context of underfixation (1-3 hours with formalin) which is typically a less discrete nuclear bubbling. This may be remedied for tissues fixed 3 hours with changed cell conditioning/protease treatment, but for those tissues fixed 1 hour are probably beyond remedy.

Properly fixed and embedded tissues expressing the RNA, DNA or antigen will remain stable if stored in a cool location (15-25°C). The Clinical Laboratory Improvement Act (CLIA) of 1988, 42CFR493.1259 (b) requires that "The laboratory must retain slides at least ten years from the date of examination and retain specimen blocks at least two years from date of examination." 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. 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. **Warning, Product Contains Formamide.** Formamide is toxic by inhalation and moderately toxic by ingestion. It is an irritant to skin, eyes, and mucous membranes and is absorbed through the skin. It may cause harm to the unborn child. Take precautions when handling reagents. Use disposable gloves and wear suitable protective clothing when handling suspected carcinogens or toxic materials.
5. Do not use beyond the specified number of tests.
6. 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.
7. NBT reagent is toxic if ingested, inhaled or absorbed through skin. Continued exposure may cause liver and kidney damage. May cause skin, eye, mucous membrane and upper respiratory tract irritation. Systemic allergic reactions are possible in sensitive individuals. Symptoms of overexposure may include eye and skin burning, coughing, laryngitis, shortness of breath, headache, nausea, dizziness, vomiting and dermatitis.
8. BCIP reagent is toxic if ingested, inhaled or absorbed through skin. Continued exposure may cause liver and kidney damage. May cause skin, eye, mucous membrane and upper respiratory tract irritation. Systemic allergic reactions are possible in sensitive individuals. Overexposure may cause liver and kidney damage. Symptoms of overexposure may include eye and skin burning and dermatitis.
9. 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.[2,3]
10. 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.
11. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
12. 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.
13. Avoid microbial contamination of reagents as this may produce incorrect results.

14. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and method sheets of all necessary components located at dialog.roche.com.
15. Consult local and/or state authorities to determine the recommended method of disposal.
16. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
17. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

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

Table 1. Hazard information.

Hazard	Code	Statement
Danger 	H317	May cause an allergic skin reaction.
	H351	Suspected of causing cancer.
	H360D	May damage the unborn child.
	H373	May cause damage to organs through prolonged or repeated exposure.
	H412	Harmful to aquatic life with long lasting effects.
	P201	Obtain special instructions before use.
	P260	Do not breathe mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P308 + P313	IF exposed or concerned: Get medical advice/ attention.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.

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

PROCEDURE

The ISH iVIEW Blue Plus Detection Kit has been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA ancillary reagents. The staining protocols can be displayed, printed and edited according to the procedure in the instrument's User Guide. Other operating parameters for the instrument have been preset at the factory.

The procedures for staining on BenchMark IHC/ISH instruments are as follows. For more detailed instructions and additional protocol options refer to the appropriate probe or antibody method sheet or the instrument's 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

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.

QUALITY CONTROL PROCEDURE

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. Optimal laboratory practice is to include a positive control section on the same slide as the patient tissue. The positive staining tissue components are used to confirm that the reagents were applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Internal tissue controls are used at the discretion of the principal investigator and the pathologist. Control tissues should be autopsy, biopsy, or surgical specimens prepared or fixed in a manner identical to the test sections. Tissue sections fixed or processed differently from the test specimen will provide comparative controls for all reagents and method steps affected by fixation and tissue processing.

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

See appropriate probe method sheet for specific positive tissue control recommendations.

Negative Tissue Control

A negative specimen control must be run with every staining procedure performed. The purposes is to monitor unintended probe and antibody cross reactivity to cellular components. The same specimen used for the positive specimen control may also be used as the negative specimen control. The variety of different cell types present in most specimens offers internal negative control sites, but this should be verified by the user. The non-staining components should demonstrate absence of specific staining and provide an indication of background staining. If unacceptable staining occurs in the negative specimen control sites, result with the patient specimens should be considered invalid.

Positive Reagent Control

Positive reagent control should be run during assay verification and troubleshooting since DNA, RNA or antigen accessibility may vary depending on fixation method and pretreatment of the specimen.

Negative Reagent Control

Negative reagent control must be substituted for the ISH probe or DNP labeled antibody with every specimen stained to aid in interpretation of each patient result. This provides an indication of nonspecific background staining for each slide. In place of an ISH probe, stain the slide with VENTANA ISH Negative Control. The incubation period for controls should correspond to that of the probe. In case of an antibody, stain the slide with VENTANA Negative Control (Polyclonal).

The negative control is especially important with the finding that the intestinal form of alkaline phosphatase may be found in cells other than the brush border of intestinal epithelial cells. Additionally, enzymes capable of reducing nitro blue tetrazolium may be preserved during fixation.

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 The performance of the ISH iVIEW Blue Plus Detection Kit was evaluated through reproducibility and other relevant studies. All staining was performed using the protocol as noted in the probe method sheet on BenchMark IHC/ISH instruments unless otherwise specified.

SUMMARY OF EXPECTED RESULTS

VENTANA ISH iVIEW Blue Plus Detection Kit, ISH probes, DNP labeled antibodies and accessory reagents in conjunction with a VENTANA automated slide stainer will result in blue precipitate at the site of the target sequence or antigen.

Specificity and sensitivity of target detection have been optimized and tested by Ventana. However, each ISH probe and labeled antibody must be validated by user to ensure desired staining.

Intra-run reproducibility of staining with VENTANA ISH *i*VIEW Blue Plus Detection Kit was determined by staining 60 HPV 3 in 1 System Control Slides: 16/18/Neg using INFORM HPV II Family 16 Probe (5 slides per run for multiple runs), 10 cervical tissue slides using INFORM HPV II Family 6 Probe and 10 cervical tissue slides using Alu Positive Control Probe II on BenchMark IHC/ISH instruments. All slides stained with comparable intensity.

Users should verify intra run (within run) reproducibility results by staining several sets of serial sections with low, medium, and high target sequences in a single run.

Inter-run reproducibility of staining with VENTANA ISH *i*VIEW Blue Plus Detection Kit was determined by staining cervical tissues and control cell lines, using INFORM HPV II Family 16 and Family 6 probes as well as Alu Positive Control Probe II on 2 BenchMark and 2 BenchMark XT automated slide stainers, 3 runs on each instrument. Greater than 90% of slides stained with comparable intensity. Users should verify inter run (between run) reproducibility results by staining several sets of serial sections with low, medium, and high target sequences on different instrument runs.

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 the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist,[4] or the CLSI Approved Guideline[5] or both documents). 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 ISH *i*VIEW Blue Plus Detection Kit causes a blue colored reaction product to precipitate at the target nucleic acid sequence or antigen bound by the probe or antibody. A qualified pathologist who is experienced in ISH/IHC procedures must evaluate controls and qualify the stained product before interpreting results. Staining of negative controls must be noted first, and these results compared to the stained material to verify that the signal generated is not the cause of nonspecific interactions.

Controls

The stained positive reagent and specimen controls should be examined first to ascertain that all reagents are functioning properly. The presence of a blue colored reaction product within the target cells is indicative of positive reactivity.

The negative reagent and specimen controls should be examined after the positive control to verify the specificity of the reaction. There should be no specific staining in the negative control. If staining occurs, it may indicate non-specific cross reactivity to cells or cellular components. Intact cells should be used for interpretation of staining results since necrotic or degenerated cells often stain nonspecifically.

If the positive or negative controls fail to demonstrate appropriate staining, any results with the test specimens should be considered invalid.

Patient Specimen

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. A negative result means that the DNA or RNA sequence or the antigen in question was not detected, not necessarily that they are absent in the cells assayed. The morphology of each sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any ISH/IHC result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

LIMITATIONS

General Limitations

1. ISH and IHC are multiple step methodologies that require specialized training in the selection of the appropriate reagents, specimen preparation, processing, preparation of the slide, and interpretation of the 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, reagent trapping, or false negative or false positive results.
3. Inconsistent results may be a consequence of variations in fixation and embedding methods, or inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the reagents and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for the review of the stained slides and assuring the adequacy of controls.
6. Ventana provides reagents at optimal dilution for use when the provided instructions are followed. Further dilution may result in loss of appropriate staining; the user must validate any such change. 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.
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 tissues. Contact your local support representative with documented unexpected reactions.
8. Due to variation in specimen processing it may be necessary to increase or decrease the ISH protease treatment time on individual specimens. Such changes must be validated by the user. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

Specific Limitations

1. Each step of the detection kit procedure has been optimized on BenchMark IHC/ISH instruments and is preset. Because of variation in tissue fixation and processing, it may be necessary to increase or decrease the hybridization time on individual specimens. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances"[6] or "Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist."[7]
2. The detection kit, in combination with Ventana probes and accessories, detects nucleic acid sequence and antigens that survives routine formalin fixation, tissue processing, and sectioning.
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 or antigen was absent in the cells or tissue assayed.
4. This detection kit has been optimized for use with VENTANA Reaction Buffer wash solution, primary antibodies, accessories, and BenchMark IHC/ISH 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), also known as LCS or Liquid Coverslip (High Temperature). 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 VENTANA automated slide stainers.
6. Certain endometrial tissues may demonstrate false positive staining when stained with the ISH *i*VIEW Blue Plus Detection Kit. Use of the ISH Negative Control Reagent is required to detect this staining.

PERFORMANCE CHARACTERISTICS

The performance of the ISH *i*VIEW Blue Plus Detection Kit was evaluated through reproducibility and other relevant studies. All staining was performed using the protocol as noted in the probe method sheet on BenchMark IHC/ISH instruments unless otherwise specified.

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TROUBLESHOOTING

1. Refer to the Troubleshooting section of the appropriate probe/antibody method sheet.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label.
3. If the positive control is negative or exhibits weaker staining than expected, other positive controls stained on the same staining run should be checked to determine if the failure is due to the control slide or reagents used. Specimens that have been improperly collected, fixed, stored, or deparaffinized may not exhibit appropriate staining.
4. If the slides demonstrate whiskering artifact from the detection chemistry, dehydration is not complete and the dehydration process should be followed by putting the slides through 2 changes of clean acetone.
5. If excessive background staining occurs with the *i*VIEW detection kits, high levels of endogenous biotin may be present. Add a biotin blocking step to the staining protocol and use the Endogenous Biotin Blocking Kit.
6. If all of the paraffin has not been removed, there may be no staining. The deparaffinization procedure should be repeated.
7. 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.
8. If sections thicker than 4 µm exhibit nuclear bubbling due to excess paraffin, select the “extended deparaffinization” option in the staining procedure.
9. For corrective action, refer to the Instructions For Use section, the instrument’s User Guide, or contact your local support representative.
10. 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 760-097 for information about proper use.

REFERENCES

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3. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
4. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2001.
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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
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
G	Updates to Material Provided section

INTELLECTUAL PROPERTY

CONTACT INFORMATION

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

www.roche.com