VENTANA Magenta ISH DIG Detection Kit

REF 760-514

08507201001

IVD Σ 60

INTENDED USE

The VENTANA Magenta ISH DIG Detection Kit is an indirect detection system for detecting DIG-labeled targets. The kit is intended to identify targets by chromogenic magenta *in situ* hybridization (ISH) in sections of formalin-fixed, paraffin-embedded tissue that are stained on a BenchMark IHC/ISH instrument.

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

The reagent is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

In general, ISH uses labeled probes to detect specific DNA or RNA target sequences in fixed tissue sections. Target nucleic acid sequences are exposed through heat-driven denaturation of nucleic acids. The reaction is then cooled to allow labeled nucleic acid probes to hybridize to complementary nucleic acid sequences.

The indirect detection technique used by this kit utilizes an antibody directed against a hapten-labeled probe, an HRP based tyramide amplification step, and an HRP mediated substrate-chromogen system (see Figure 1).

PRINCIPLE OF THE PROCEDURE

Following probe hybridization and stringency washes, target-bound digoxigenin (DIG) labeled probe is detected with HRP-labeled anti-DIG antibody. Signal amplification is accomplished through HRP catalyzed oxidation of tyramide-linked DIG by H_2O_2. The reactive oxidized form of tyramide covalently binds to tyrosine residues, increasing the local concentration of DIG hapten. This additional bound DIG is detected through a second application of the HRP labeled anti-DIG antibody. Finally, signal is visualized through another tyramide reaction involving tyramide-linked sulforhodamine B magenta dye.

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 instrument washes the sections to remove unbound material and applies a liquid coverslip to minimize 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.

For more detailed information on instrument operation, refer to the appropriate User Guide.

MATERIAL AND METHODS

Material Provided

The VENTANA Magenta ISH DIG Detection Kit (VENTANA DIG Detection Kit) contains sufficient reagent for 60 tests.

One 24 mL dispenser	VENTANA Magenta ISH DIG HRP contains an HRP labeled anti-DIG mouse monoclonal antibody (~1.0µg/mL) in a MOPS buffered protein solution with ProClin preservative.
One 12 mL dispenser	VENTANA Magenta ISH DIG Tyramide Amplifier contains a DIG labeled tyramide (~4.01µg/mL) in an aqueous buffer with ProClin preservative (~10% C_2H_6OS).
One 12 mL dispenser	VENTANA Magenta ISH DIG Tyramide SRB Chromogen contains an SRB labeled tyramide (~295µg/mL) in an aqueous buffer with ProClin preservative (~20% C_2H_6OS).

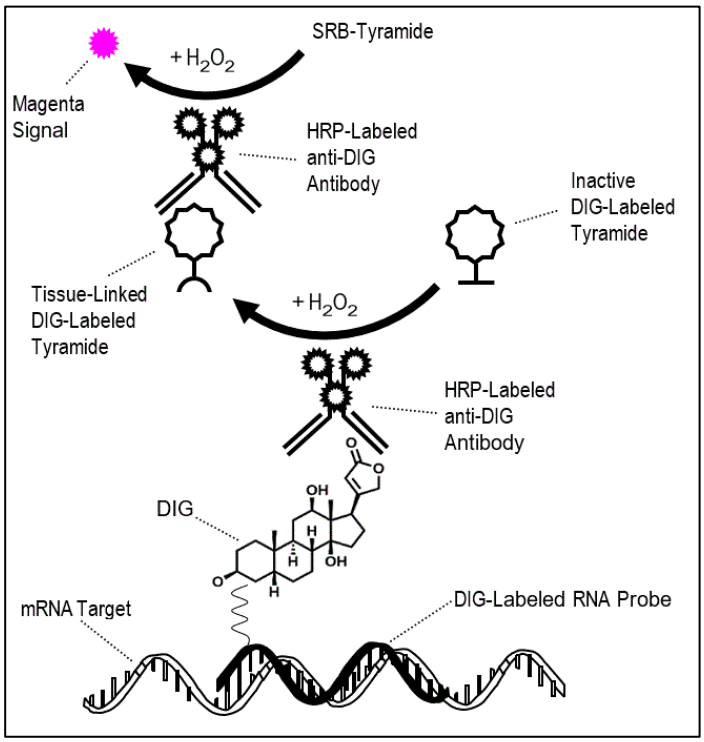

Figure 1. VENTANA Magenta ISH DIG Detection Kit

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 reduction of stain quality.

Materials Required but Not Provided

Staining reagents, such as probe and ancillary components, 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. ISH DIG-labeled probe
2. VENTANA Silver ISH BF Detection Kit (Cat. No. 760-513 / 08507031001)
3. ISH TSA Ancillary Kit (Cat. No. 760-515 / 08507082001)
4. ISH Peroxidase Inhibitor (Cat. No. 780-5061 / 07729014001)
5. HybReady Solution (Cat. No. 780-4409 / 05917557001)
6. ISH Protease 3 (Cat. No. 780-4149 / 05273331001)
7. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
8. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
9. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
10. SSC (10X) (Cat. No. 950-110 / 05353947001)
11. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
12. ULTRA CC1 (Cat. No. 950-224 / 05424569001)
13. ULTRA CC2 (Cat. No. 950-223 / 05424542001)
14. ULTRA LCS (Cat. No. 650-210 / 05424534001)
15. *ultra*View Silver Wash II (Cat. No. 780-003 / 05446724001)
16. Microscope slides, Superfrost™ Plus
17. BenchMark IHC/ISH instrument
18. General purpose laboratory equipment

Storage and Stability

Upon receipt and when not in use, store at 2-8°C. Do not freeze. This kit can be used immediately after removal from the refrigerator.

To ensure proper reagent delivery and stability of the kit components, replace the dispenser caps after every use and immediately place the dispensers in the refrigerator in an upright position.

Every kit 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 Collection and Preparation for Analysis

Routinely processed formalin-fixed, paraffin-embedded tissues are suitable for use with the VENTANA DIG Detection Kit. The recommended tissue fixative is 10% neutral buffered formalin (NBF) for 6-72 hours[1].

Specimens should be cut to 4µm sections and placed on positively charged microscope slides (Superfrost™ Plus). Slides should be drained or dried to remove excess water between slide and tissue prior to BenchMark IHC/ISH instrument staining. Variable results may occur as a result of tissue section thickness, fixation type, incomplete prolonged fixation or special processes such as decalcification of bone marrow preparations.

Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining of any ISH assay (for example, lack of staining or counterstain on the tissue). Ask your Roche representative for a copy of "Impact of environmental stress on various histology slide types" to better understand how to use these types of slides.

It is recommended that any control slides be run simultaneously with unknown specimens. Note that the magenta signal will slowly change hue over time with long term exposure to light. This should not impact normal clinical practices, but slides should be stored out of direct light when not in use.

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[2,3].
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 IHC/ISH instrument User Guide and method sheets of all necessary components located at dialog.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.

This detection kit 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.
	P261	Avoid breathing mist or vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

Contains Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1). May produce allergic reaction.

STAINING PROCEDURE

The VENTANA DIG 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 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 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

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 two sequential solutions of a mild dishwashing detergent (do not use detergent designed for automatic dishwashers).
2. Rinse slides well with distilled water for approximately about 1 minute. Shake off excess water.
3. Transfer the slides to a 90% ethanol bath for approximately 1 minute.
4. Transfer the slides to a 100% ethanol bath for approximately 1 minute.
5. Transfer the slides to a second 100% ethanol bath for approximately 1 minute.
6. Transfer the slides to a xylene bath for approximately 1 minute.
7. Transfer the slides to a second xylene bath for approximately 1 minute.
8. Place coverslip on slide. Note that some mounting media are not compatible with the VENTANA DIG Detection Kit (see the Limitations section).

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

If applicable, see appropriate probe method sheet.

Positive Reagent Control

If applicable, see appropriate probe method sheet.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local support representative immediately. Patient results are invalid if quality control results do not meet specifications. Identify and correct the problem, then repeat the patient sample (refer to Troubleshooting).

Assay Verification

Prior to initial use of a reagent in a diagnostic procedure, the performance of the reagent should be verified by testing it on a series of specimens with known ISH performance characteristics (refer to the probe method sheet, the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program Anatomic Pathology Checklist[4], and the CLSI Approved Guideline[5]). These quality control procedures should be repeated for each new lot of reagent, or whenever there is a change in assay parameters.

INTERPRETATION OF RESULTS

The VENTANA DIG Detection Kit causes a magenta dye to covalently link to tyrosine residues near the nucleic acid sequence hybridized to by the probe. A qualified pathologist who is experienced in ISH procedures must evaluate controls and qualify the stained product before interpreting results.

LIMITATIONS

General Limitations

1. ISH is a multiple step methodology that requires 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. 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 staining 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.
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. 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.

Specific Limitations

1. The VENTANA DIG Detection Kit was developed to be used with tissues cut to 4µm thickness. Sections thinner/thicker than this may experience inappropriate staining and/or tissue loss.
2. Not all mounting media may be compatible with the magenta chromogen. Refer to Table 2 for mounting media that have been tested.
3. Stained slides should be stored in the dark when not in use to prevent hue shift of the magenta chromogen.
4. The detection kit, in combination with VENTANA probes and accessories, detects nucleic acid sequence that survives routine formalin fixation, tissue processing, and sectioning. As with any test, a negative result means that the specific target was not detected in the tissue sample and not that the target was absent in the original, unfixed tissue.
5. This detection kit has been optimized for use with VENTANA reagents on BenchMark IHC/ISH instruments. Users who deviate from recommended test procedures are responsible for interpretation of patient results under these circumstances.
6. All detection kits might not be registered on every instrument. Please contact your local support representative for more information.

Table 2. Compatibility of mounting media.

Mounting Media	Manufacturer	Compatible?
Acrytol	Electron Microscopy Sciences	Yes
Consul-Mount	Epredia	Yes
Cytoseal XYL	Richard Allan Scientific	Yes
Cytoseal 60	Richard Allan Scientific	Yes
Dako Mounting Medium	Dako	Yes
Diamount	Diapath	Yes
DPX Mountant	CDH	Yes
Entellan	Merck	Yes
Glycergel	Dako	Yes
HE600	Roche	Yes
HistoCore Spectra CV X1	Leica	Yes
Histomount	National Diagnostics	Yes
MicroMount	Leica	Yes
Canada Balsam	Elabscience	Yes
Permount	Electron Microscopy Sciences	Yes
Pertex	Histolab	Yes
Epredia Synthetic Mountant	Epredia	Yes
Sub-X Mounting	Leica	Yes
Tissue-Tek Film	Sakura	Yes
Entellan New	Merck	No
Eukitt	Merck	No

PERFORMANCE CHARACTERISTICS

For details regarding performance characteristics, refer to the appropriate probe method sheet.

TROUBLESHOOTING

1. Ensure that the section was cut to 4µm and a compatible mounting media was used.
2. Ensure that reagent dispensers are not clogged or empty. Test dispenser function by aiming the dispenser over a waste container and firmly pressing down on top of the barrel, ensuring that a single drop is dispensed. If the dispenser is clogged or not dispensing properly, contact your support representative, and do not use the dispenser.
3. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
4. Refer to the Troubleshooting section of the appropriate probe method sheet for assay specific troubleshooting solutions.
5. For corrective action, refer to the STAINING procedure section, the instrument User Guide, or contact your local support representative.

REFERENCES

1. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
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, 2007.
5. CLSI (formerly NCCLS). Quality Assurance for Design Control and Implementation of Immunocytochemistry Assays: Approved Guideline-Second Edition. CLSI document I/LA28-A2 (ISBN 1-56238-745-6). CLSI, 950 West Valley Road, Suite 2500, Wayne, PA 19087-1898 USA, 2011.

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 dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identifier
	Indicates the entity importing the medical device into the European Union

INTELLECTUAL PROPERTY

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