VENTANA Silver ISH DNP Detection Kit

REF 760-516

08318883001

IVD Σ 60

INTENDED USE

The VENTANA Silver ISH DNP Detection Kit is an indirect system for detecting DNP-labeled targets. 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 BenchMark IHC/ISH instruments.

This product should be interpreted by a qualified reader 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 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 a secondary antibody directed against the hapten 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 probe binding. VENTANA Silver ISH DNP Detection Kit uses the indirect method to visualize complementary nucleic acid sequences by depositing a black colored precipitate.

PRINCIPLE OF THE PROCEDURE

VENTANA Silver ISH DNP Detection Kit detects DNP-labeled probes bound to a specific sequence in formalin-fixed, paraffin-embedded (FFPE) tissue sections. The detection kit contains a primary antibody and an enzyme-labeled secondary antibody conjugated to horseradish peroxidase (HRP) which is used as the chromogenic enzyme. During the ISH staining process, DNP probes are co-hybridized to their respective specific target DNA sequences within the cell nuclei. VENTANA Silver ISH (SISH) DNP Detection Kit contains the following dispensers: mouse anti-DNP primary antibody labeled with hydroxyquinoxaline (HQ), mouse anti-HQ secondary antibody conjugated to horseradish peroxidase (HRP), Chromogen A (Silver A), Chromogen B (Silver B) and Chromogen C (Silver C). Following incubation with the HQ-labeled mouse anti-DNP primary antibody and then mouse anti-HQ HRP secondary antibody conjugate, the SISH reaction occurs. Briefly described, this reaction is driven by the sequential addition of Chromogens A (silver acetate), B (hydroquinone) and C (H_2O_2). Here, the silver ions (Ag^+) are reduced by hydroquinone to metallic silver atoms (Ag^0). This reaction is fueled by the substrate for HRP, hydrogen peroxide (Chromogen C). Figure 1 illustrates the SISH reaction. The specimen is then counterstained with Hematoxylin II for interpretation 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.

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

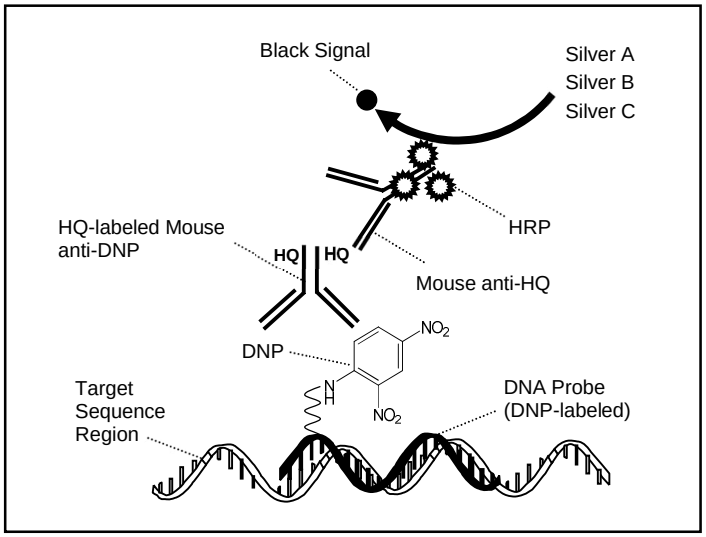

Figure 1. VENTANA Silver ISH DNP Detection

MATERIAL AND METHODS

Materials Provided

The VENTANA Silver ISH DNP Detection Kit contains sufficient reagent for 60 tests.

One 6 mL dispenser	VENTANA Silver ISH DNP HQ reagent contains an anti-DNP hapten-labeled antibody (~12.5 µg/mL) in a protein and phosphate containing buffer with 0.05% ProClin 300 solution, a preservative.
One 6 mL dispenser	VENTANA Silver ISH DNP HQ HRP reagent contains an anti-HQ horseradish peroxidase (HRP) enzyme conjugate antibody (~25 µg/mL) solution in a protein and phosphate containing buffer including 0.05% ProClin 300 solution, a preservative.
One 12 mL dispenser	VENTANA Silver ISH DNP Chromogen A reagent contains approximately 1% CH_3COOAg in an aqueous solution.
One 6 mL dispenser	VENTANA Silver ISH DNP Chromogen B reagent contains approximately 1% $C_6H_6O_2$ in an aqueous solution.
One 6 mL dispenser	VENTANA Silver ISH DNP Chromogen C reagent contains approximately 0.2% H_2O_2 in an aqueous solution.

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.

Materials Required but Not Provided

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

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

The following reagents and materials may be required for staining but are not provided with the detection kit:

1. ISH probe
2. ISH Protease 3 (Cat. No. 780-4149 / 05273331001)
3. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
4. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
5. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
6. SSC (10X) (Cat. No. 950-110 / 05353947001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)

8. *ultra*View Silver Wash II (Cat. No. 780-003 / 05446724001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. Cell Conditioning Solution (CC2) (Cat. No. 950-123 / 05279798001)
11. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
12. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. ULTRA Cell Conditioning Solution (ULTRA CC2) (Cat. No. 950-223 / 05424542001)
14. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
15. BenchMark IHC/ISH instrument
16. Superfrost Plus microscope slides, positively charged
17. Mounting medium*
18. Automated coverslipper
19. General purpose laboratory equipment

* See Table 2 for compatible mounting media with this assay.

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

FFPE tissues are suitable for use with the VENTANA Silver ISH DNP Detection Kit and BenchMark IHC/ISH instruments (see Materials Required but Not Provided section). The recommended tissue fixative is 10% neutral buffered formalin (NBF)[1] for 6 to 72 hours. 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. 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.

Each section should be cut to the appropriate thickness (~4 µm) for the probe being used and placed on a positively charged glass microscope slide. Slides should be drained or dried to remove excess water between slide and tissue.

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 interfere with signal enumeration. However, severe cases of nuclear bubbling may distort the nuclei or SISH 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, (see Troubleshooting). Nuclear bubbling also may occur in the context of under-fixation (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 those tissues fixed 1 hour are probably beyond remedy.

Refer to the appropriate probe method sheet for cut slide stability and environmental storage conditions.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. ProClin 300 solution is used as a preservative in this 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. Materials of human or animal origin should be handled as potentially biohazardous and disposed of with proper precautions.
5. 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]
6. 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.
7. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
8. 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.
9. Avoid microbial contamination of reagents as this may produce incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
11. Consult local and/or state authorities to determine the recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This 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.

EUH208: Contains reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1), hydroquinone. May produce an allergic reaction.

PROCEDURE

The VENTANA Silver ISH DNP 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 dispenser, appropriate detection kit dispensers, and required accessory reagent dispensers onto the reagent tray and place them on the instrument.
3. Check bulk fluids and empty waste.
4. The reaction buffer bulk bottles must be full.
5. The waste container must be empty prior to the start of the run.
6. Load the slides onto the instrument.
7. Start the staining run.
8. At the completion of the run, remove slides from the instrument. The stained slides will have residual buffer and liquid coverslip solution on them. Proceed with rinsing and dehydration (see below).

Recommended Post-Instrument Processing Procedures

1. To remove liquid coverslip solution, wash 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. Place slides in an oven (45-60°C) to dry or air dry at ambient temperature. In an oven, drying times range from 10 minutes to one hour (drying stained slides for a longer period of time does not appear to impact staining results). Ensure slides are completely dry before coverslipping, as residual water on the slides can interfere with the coverslipping procedure and cause bubbles to form.
4. Transfer slides into xylene bath for approximately 30 seconds.
5. Place mounting media on slide.
6. Place coverslip on slide. Note that some mounting media are not compatible with the assay and should not be used (See Limitations and Troubleshooting sections).

Quality Control Procedures

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 qualified reader. 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. 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 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

VENTANA Silver ISH DNP Detection Kit causes a black (silver) colored reaction product to precipitate at the nucleic acid sequence hybridized by the probe. A qualified reader who is experienced in ISH procedures must evaluate controls and qualify the stained slides 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 result of nonspecific interactions. Refer to the Interpretation of Results section of the appropriate probe method sheet.

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 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 qualified 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. Tissue sections should be cut at ~4 µm in thickness. Sections thicker than 4 µm may experience tissue loss.
2. Refer to the appropriate assay method sheet for optimized staining procedure.
3. The detection kit, in combination with VENTANA probes and accessories, detects nucleic acid sequence that survives routine formalin fixation, tissue processing, and sectioning.
4. As with any test, a negative result means that the specific target was not detected, not that the specific target was absent in the cells or tissue assayed.
5. This detection kit has been optimized for use with Reaction Buffer wash solution, probes, 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.
6. 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 in situ hybridization (ISH) reactions carried out on BenchMark IHC/ISH instruments.
7. Oxidation, fading, and/or disappearance of the SISH signal may be due to certain brands of mounting media. See Table 2 for compatibility of mounting media.
8. All detection kits might not be registered on every instrument. Please contact your local Roche representative for more information.

Table 2. Compatibility of Mounting Media with SISH based assays.

Mounting Media	Manufacturer	Type	Compatibility with SISH
Entellan	Merck	Xylene	No
Entellan New	Merck	Xylene	No
Eukitt	EMS	Xylene	No
HSR	Sysmex	Xylene	No
Malinol	Muto Chemical	Xylene	No
Acrytol	SurgiPath	Xylene	Yes
Alcolmount	Diapath	Alcohol	Yes
BioMount 2	BBInternational	Xylene	Yes
Cytoseal 60	Richard Allan Scientific	Xylene	Yes
Diamount	Diapath	Xylene	Yes

Mounting Media	Manufacturer	Type	Compatibility with SISH
DPX	BDH: Raymond Lamb	Xylene	Yes
FloTexx	Lerner Labs	Xylene	Yes
Gel Mount	Biomeda	Aqueous	Yes
Histomount	Raymond Lamb	Xylene	Yes
MicroMount	SurgiPath	Xylene	Yes
MM24	SurgiPath	Xylene	Yes
Mountex	Histolab	Xylene	Yes
MountQuick	Daido Sangyo Co.	Aqueous	Yes
Paramount	Protaqs Quartett: Dako	Xylene	Yes
Permount	Fisher	Xylene	Yes
Pertex	Cell Path	Xylene	Yes
Shandon Consul mount	Thermo Scientific	Xylene	Yes
Softmount	WAKO	Lemasol A	Yes
SureMount	Triangle Biomedical Sciences	Xylene	Yes
Thermo EZ Mount	Thermo Scientific	Xylene	Yes
Ultramount	Dako	Xylene	Yes

PERFORMANCE CHARACTERISTICS

The performance of the VENTANA Silver ISH DNP 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.

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

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.
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. For corrective action, refer to the Procedure section, the instrument User Guide, or contact your local support representative.

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 inline dispenser method sheet associated with P/N 760-516 for more information.

REFERENCES

1. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
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 24 June 2020 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 elabdoc.roche.com/symbols for more information).

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

REVISION HISTORY

Rev	Updates
E	Updates to Warnings and Precautions section. Administrative updates, no change to content.

INTELLECTUAL PROPERTY

For USA: Rx only

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