VENTANA U6 BF Probe

REF 760-7062

08773866001

IVD Σ 30

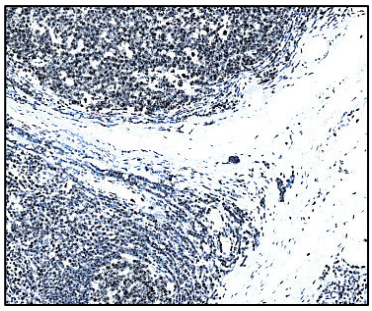

Figure 1. U6 snRNA expression pattern in tonsil.

INTENDED USE

The VENTANA U6 BF Probe is intended to assess RNA integrity 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

Because RNA is susceptible to degradation, the ubiquitously expressed U6 snRNA transcript is commonly used as a surrogate to assess RNA integrity. The VENTANA U6 BF Probe is used to evaluate RNA integrity in patient cases where loss of RNA is suspected.

The VENTANA U6 BF Probe is a benzofurazan hapten (BF) labeled 2'-O-Methyl oligonucleotide probe that spans approximately 80 bases of the U6 snRNA transcript. U6 is visualized in black with the VENTANA Silver ISH BF Detection Kit. The presence of U6 signal indicates that RNA in a target sample is not completely degraded.

PRINCIPLE OF THE PROCEDURE

The VENTANA U6 BF Probe is optimally formulated for use with the VENTANA Silver ISH BF Detection Kit, the ISH TSA Ancillary Kit, and accessory reagents on a BenchMark IHC/ISH instrument.

During the *in situ* hybridization (ISH) staining process, BF labeled probe is hybridized to complimentary U6 snRNA in the tissue and detected with the VENTANA Silver ISH BF Detection Kit. This detection system uses HRP-labeled anti-BF mouse monoclonal antibody and BF-labeled tyramide amplification to visualize the target as black signal through the precipitation of silver (see Figure 2). Refer to the VENTANA Silver ISH BF Detection Kit method sheet for additional information.

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.

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

MATERIAL PROVIDED

Material Provided

The VENTANA U6 BF Probe contains sufficient reagent for 30 tests.

One 6 mL dispenser contains approximately 0.18 ng/mL labeled probe in a formamide-based hybridization buffer (approximately 50% CH_3NO).

Reconstitution, Mixing, Dilution, Titration

The probe is optimized for use on BenchMark IHC/ISH instruments. No reconstitution, mixing, dilution, or titration of reagent is required. Further dilution may result in reduction of stain quality.

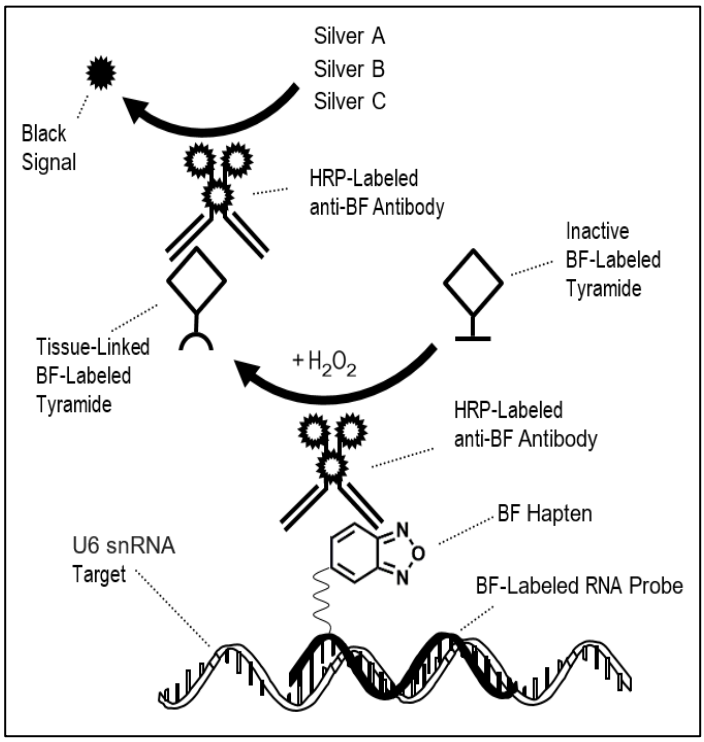

Figure 2. VENTANA Silver ISH BF Detection Kit

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits 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. VENTANA Silver ISH BF Detection Kit (Cat. No. 760-513 / 08507031001)
2. VENTANA Magenta ISH DIG Detection Kit (Cat. No. 760-514 / 08507201001)
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. ISH Negative Control (Cat. No. 780-2902 / 05272165001)
10. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
11. SSC (10X) (Cat. No. 950-110 / 05353947001)
12. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
13. ULTRA CC1 (Cat. No. 950-224 / 05424569001)
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 probe can be used immediately after removal from the refrigerator.

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

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

SPECIMEN PREPARATION

Routinely processed formalin-fixed, paraffin-embedded tissues are suitable for use with the VENTANA U6 BF Probe. 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.

Slides should be stained immediately, as quality of RNA targets in cut tissue sections may diminish over time. Internal studies have shown that cut slides stored at room temperature can be stable for at least 60 days.

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 patient specimens. Note that the silver signal may slowly fade 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. Do not use beyond the specified number of tests.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[2,3]
5. 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.
6. If reagents come in contact with sensitive areas, wash with copious amounts of water. Avoid inhalation of reagents.
7. 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.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. 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 navifyportal.roche.com.
10. Consult local and/or state authorities to determine the recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. 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 	H351	Suspected of causing cancer
	H360D	May damage the unborn child.
	H373	May cause damage to organs through prolonged or repeated exposure
	P201	Obtain special instructions before use.
	P202	Do not handle until all safety precautions have been read and understood.
	P260	Do not breathe mist or vapours.
	P280	Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
	P308 + P313	If exposed or concerned: Get medical advice/attention.
	P501	Dispose of contents/container to an approved waste disposal plant.

STAINING PROCEDURE

The VENTANA U6 BF Probe is intended to be used as an RNA integrity control as an aid in interpretation of other VENTANA RNA probes. The staining procedure used for the VENTANA U6 BF Probe should be the same as the procedure used for the probe being assessed. Refer to the appropriate probe method sheet for staining procedure and protocol guidance.

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 U6 BF Probe (see the Limitations section).

QUALITY CONTROL PROCEDURE

Negative Probe Control

ISH Negative Control (Cat. No. 780-2902 / 05272165001) may be used in place of the VENTANA U6 BF Probe to assess for detection driven background in a patient sample.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local support representative immediately.

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 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 reagents, whenever there is a change in assay parameters, or whenever there is a change in specimen preparation.

INTERPRETATION OF RESULTS

A qualified pathologist experienced in the microscopic interpretation of anatomic pathology specimens must interpret the results.

U6 target will stain black, and the typical positive staining pattern is punctate nuclear staining. The presence of U6 signal indicates that RNA in a target sample is not completely degraded.

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, antibody 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 ensuring 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. 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. Not all fixatives may be compatible with the VENTANA U6 BF Probe. Ventana recommends fixation with 10% NBF for 6-72 hours. Refer to Table 2 for fixatives that have been tested. Fixation time should be validated prior to use.
2. The VENTANA U6 BF Probe has been shown to be compatible with HCl, formic acid, and EDTA decalcification reagents, but this compatibility is highly dependent on reagent concentration and treatment time. Not all decalcification reagents may be compatible with the VENTANA U6 BF Probe. Refer to Table 3 for specific reagents that have been tested. Decalcification time should be validated prior to use.
3. The VENTANA U6 BF Probe was developed to be used with tissues cut to 4µm thickness. Sections thinner/thicker than this may experience inappropriate staining and/or tissue loss.
4. Tissues should be stained within 60 days of sectioning. Loss of staining may be observed in tissues stained after 60 days.
5. Not all mounting media may be compatible with the silver chromogen. Refer to Table 4 for mounting media that have been tested.
6. Stained slides should be stored in the dark when not in use to prevent fading of the silver chromogen.
7. The probe, in combination with VENTANA detection kits 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.
8. 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.
9. All assays might not be registered on every instrument. Please contact your local support representative for more information.

Table 2. Compatibility of fixatives.

Fixative	Compatible?
10% Neutral buffered formalin	Yes
20% Neutral buffered formalin	Yes
10% Unbuffered formalin	Yes
Bouin's	Yes
Zinc formalin	Yes
Alcoholic formalin acetic acid	No
Prefer	No
Alcoholic formalin	No
Diff-Quik	No

Table 3. Compatibility of tested decalcification reagents.

Decalcifier	Manufacturer	Type	Compatible?
Decal Decalcifier	StatLab	HCl/EDTA	Yes
Decalcifying Solution B	Fisher Scientific	HCl/EDTA	Yes
Formical 2000	StatLab	Formic acid/EDTA	Yes
Immunocal	StatLab	Formic acid	Yes

Table 4. 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

The following performance characteristics were demonstrated for the VENTANA U6 BF Probe:

1. Within-run, between-day, and between-instrument precision was conducted on the BenchMark ULTRA instrument with seven U6 positive and seven U6 negative cases. Overall percent agreements for each category were calculated with 2-sided 95% confidence intervals (CI) using the Wilson Score method: within-run (98/98, 95% CI of 96.2-100%), between-day (140/140, 95% CI of 97.3-100%), and between-instrument (84/84, 95% CI of 95.6-100% CI).
2. Sensitivity and specificity was conducted on the BenchMark ULTRA with 20 RNA positive cases and 11 RNase treated cases. Pass rate was calculated with 2-sided 95% confidence intervals using the Wilson Score method: sensitivity (20/20, 95% CI of 83.9-100%) and specificity (11/11, 95% CI of 74.1-100%).

All studies met their acceptance criteria.

TROUBLESHOOTING

Table 5. Troubleshooting solutions.

Issue	Solution
Absent or weak staining in tissues where staining is expected	3. Check that the slide has the proper barcode label and that the correct probe was selected in the protocol. 4. Ensure that the section was cut to 4µm and a compatible mounting media was used. 5. 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. 6. If other slides that were run at the same time using the same reagents and procedures stained appropriately, an unknown slide-specific failure may have occurred. Prepare a new slide and re-stain. 7. Contact your local representative for support if other slides run at the same time also produced unexpectedly inadequate staining and no obvious source of failure can be identified.
Inappropriate background	8. Ensure that the section was cut to 4µm. 9. 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 sticks in the down position or dispenses more regent than expected, contact your support representative, and do not use the dispenser. 10. Stain a slide from the patient specimen with ISH Negative Control to evaluate for detection kit associated background. Subtract this background from the VENTANA U6 BF Probe stained slide to interpret RNA integrity. 11. If background still interferes with the ability to determine RNA integrity status, a new specimen may be required.
Tissue washes off slides.	Ensure that Superfrost™ Plus slides are used.

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 border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

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
B	Updates to Warnings and Precautions Section. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

CONTACT INFORMATION

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

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany
+800 5505 6606

CE