VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail

REF 800-6054

08507023001

IVD Σ 30

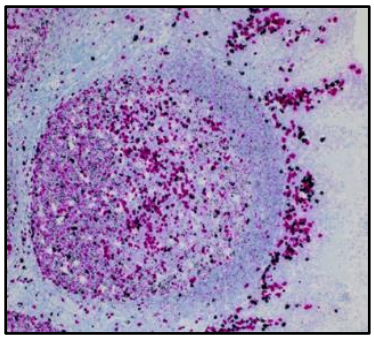


Figure 1. Kappa and lambda mRNA expression patterns in tonsil.

INTENDED USE

VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail is intended for the qualitative detection of Kappa mRNA and Lambda mRNA in formalin-fixed, paraffin-embedded (FFPE) human bone marrow and lymphoid tissue stained on a BenchMark IHC/ISH instrument using chromogenic in situ hybridization (ISH) and visualized using light microscopy. VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail is intended as an aid in the identification of B-cell lymphomas and plasma cell neoplasms.

Results of the assay 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

Evaluation of B-cell clonality is a useful aid in the diagnosis of suspected B-cell and plasma cell neoplasms. A commonly used method for determining B-cell clonality involves the assessment of kappa and lambda light chain expression in FFPE tissue. However, expression levels of kappa and lambda light chains in normal B-cells and B-cell neoplasms depend greatly on the stage of differentiation, and many available assays have limited utility due to insufficient sensitivity to the lower ranges of expression.

VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail (VENTANA K/L Probe Cocktail) is intended to provide sensitive and dynamic detection for both kappa and lambda light chain mRNA on a single FFPE slide, expanding the clinical utility to B-cells in all stages of maturation and their neoplastic counterparts.

VENTANA K/L Probe Cocktail is a mix of digoxigenin (DIG) and benzofurazan (BF) hapten labeled 2′-O-methyl oligonucleotide probes, each of which spans approximately 80 bases of either the kappa or lambda light chain region of the associated mRNA transcripts. The kappa target is visualized in magenta with VENTANA Magenta ISH DIG Detection Kit, and the lambda target is visualized in black with VENTANA Silver ISH BF Detection Kit. Restriction status is determined by assessing the ratio of kappa to lambda signal.

PRINCIPLE OF THE PROCEDURE

VENTANA K/L Probe Cocktail is formulated for use with the VENTANA Magenta ISH DIG Detection Kit, the VENTANA Silver ISH BF Detection Kit, the ISH TSA Ancillary Kit, and accessory reagents on a BenchMark IHC/ISH instrument.

The detection kits contain HRP-labeled anti-hapten primary antibody, hapten-labeled tyramide amplification reagent, and silver or magenta chromogen. During the ISH staining process, BF and DIG-labeled probes are hybridized to their respective target sequences in the tissue.

DIG-labeled kappa probe is detected with VENTANA Magenta ISH DIG Detection Kit. This detection system uses HRP-labeled anti-DIG mouse monoclonal antibody and DIG-labeled tyramide amplification to visualize the target as a magenta signal through the covalent linkage of sulforhodamine B labeled tyramide to the tissue (see Figure 2).

BF-labeled lambda probe is detected with 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 a black signal through the precipitation of silver (see Figure 3).

Refer to the method sheets of these detection reagents 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.

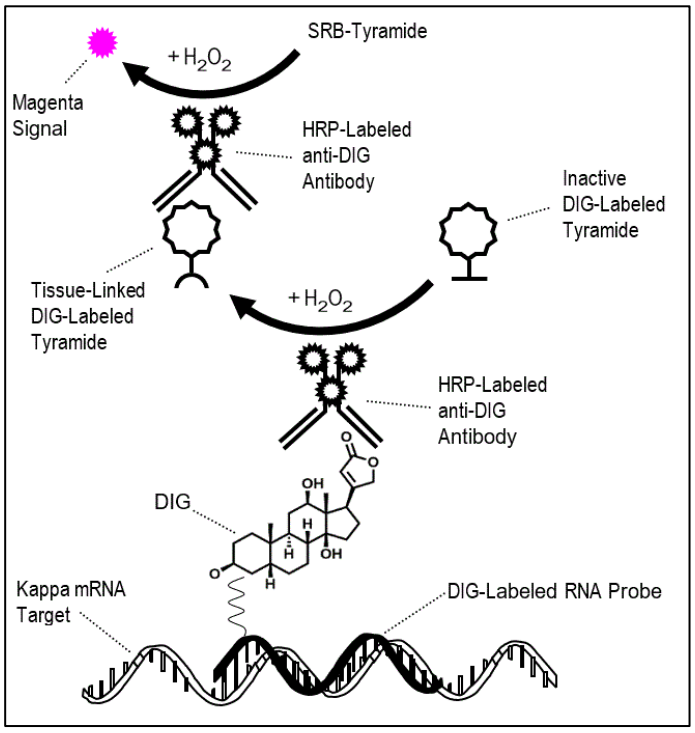


Figure 2. VENTANA Magenta ISH DIG Detection Kit

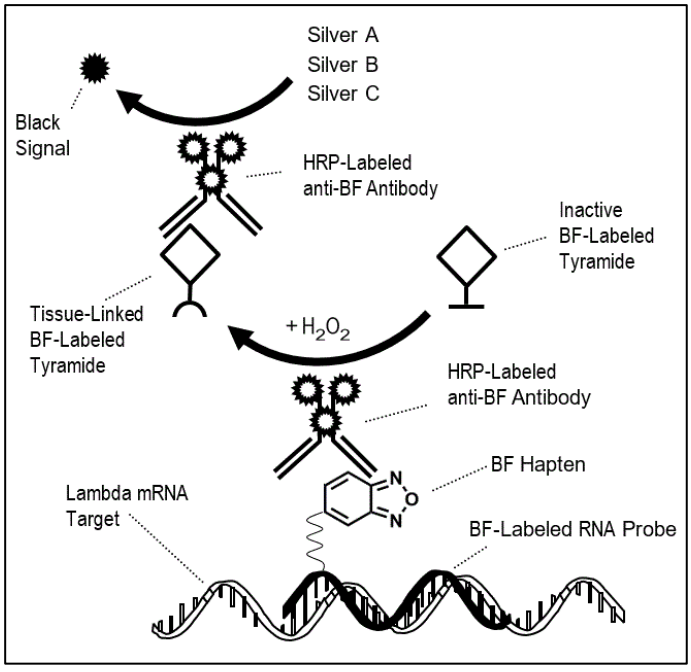


Figure 3. VENTANA Silver ISH BF Detection Kit

MATERIAL AND METHODS

Material Provided

The VENTANA K/L Probe Cocktail contains sufficient reagent for 30 tests.

One 6 mL dispenser contains approximately 0.120 µg/mL labeled probe in a formamide-based hybridization buffer (approximately 50% CH3NO).

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.

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 U6 BF Probe (Cat. No. 760-7062 / 08773866001)
2. ISH Negative Control (Cat. No. 780-2902 / 05272165001)
3. VENTANA Silver ISH BF Detection Kit (Cat. No. 760-513 / 08507031001)
4. VENTANA Magenta ISH DIG Detection Kit (Cat. No. 760-514 / 08507201001)
5. ISH TSA Ancillary Kit (Cat. No. 760-515 / 08507082001)
6. ISH Peroxidase Inhibitor (Cat. No. 780-5061 / 07729014001)
7. HybReady Solution (Cat. No. 780-4409 / 05917557001)
8. ISH Protease 3 (Cat. No. 780-4149 / 05273331001)
9. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
10. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
11. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
12. SSC (10X) (Cat. No. 950-110 / 05353947001)
13. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
14. ULTRA CC1 (Cat. No. 950-224 / 05424569001)
15. ULTRA LCS (Cat. No. 650-210 / 05424534001)
16. *ultra*View Silver Wash II (Cat. No. 780-003 / 05446724001)
17. Microscope slides, *Superfrost™ Plus*
18. BenchMark IHC/ISH instrument
19. 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 Collection and Preparation for Analysis

Routinely processed FFPE tissues are suitable for use with VENTANA K/L Probe Cocktail. The recommended tissue fixative is 10% neutral buffered formalin (NBF) for 6-72 hours[1]. For tissues that require a decalcification step, VENTANA K/L Probe Cocktail 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 probe. Refer to Table 5 and Table 6 for specific fixative and decalcification reagents that have been tested. Treatment time should be validated prior to use.

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 magenta and silver signals may slowly change hue or 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. **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. 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]
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 method sheets 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 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

VENTANA KL Probe Cocktail has been developed for use on a BenchMark IHC/ISH instrument in combination with VENTANA detection kits and accessories. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide.

The procedure "U Kappa Lambda DISH PRB-CKT" is used for staining on the BenchMark ULTRA and ULTRA Plus instruments.

Table 2. Recommended staining protocol conditions. "Short Pre-Treatment" may be selected based on individual specimens or reader preference. "Short Pre-Treatment" will reduce intensity of all specific signal, including that for nuclear Immunoglobulin Lambda Like Polypeptide 5 (IGLL5, see "INTERPRETATION OF RESULTS" section).

Staining Selectable	Setting
Short Pre-Treatment	Not Selected

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 reagents 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 K/L Probe Cocktail (see the Limitations section).

QUALITY CONTROL PROCEDURE

Positive Control Tissue

It is recommended that a laboratory-specific tonsil control tissue should be included on each patient slide to ensure that the assay is performing as expected. Normal tonsil exhibits the full range of kappa and lambda expression, whereas the stroma serves as a negative staining element. Markedly diminished signal or excessive background indicates that an error may have occurred on that slide, and the slide should not be evaluated.

Table 3. Scoring criteria for evaluation of control tonsil tissue staining.

Staining Element	Acceptable Staining	Unacceptable Staining
Known positive	Punctate cytoplasmic dot staining of almost all B-cells in the mantle zone, clearly visible at 10X, with physiologic K/L ratio (2-3:1)	Markedly diminished staining in the majority of B-cells in the mantle zone, requiring 20X for visualization
Known negative	Absent, or low level randomly scattered staining in the stroma	Excessive non-specific background staining of the squamous epithelium or stroma obscuring the enumeration of the K/L ratio

RNA Integrity Marker

The VENTANA K/L Probe Cocktail assay may have reduced performance in tissues where mRNA integrity has been impacted. Because RNA is susceptible to degradation, the ubiquitously expressed U6 transcript is commonly used as a surrogate to assess target degradation in tissue samples. While not required for interpretation of restriction status, VENTANA U6 BF Probe (Cat. No. 760-7062 / 08773866001) may be used to evaluate RNA integrity on patient cases where insufficient signal is present on the K/L slide. Negative staining with VENTANA U6 BF Probe indicates that a new patient sample may be necessary.

Patient samples stained with VENTANA U6 BF Probe should be run with the same staining procedure and pre-treatment selectable as were used for VENTANA K/L Probe Cocktail testing.

Negative Probe Control

ISH Negative Control (Cat. No. 780-2902 / 05272165001) may be used in place of VENTANA K/L Probe Cocktail to assess for detection-driven background in a patient sample. Use of a negative probe control is not required for interpretation of restriction status.

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. Identify and correct the problem, then repeat the patient samples (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 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 evaluate the control(s) before interpreting results. Refer to "Interpretation Guide for VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail for B-cell Lymphomas and Plasma Cell Neoplasms" (Cat. No. 102202118EN) for additional resources to aid in the evaluation of patient and control staining. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

With the assay, kappa targets will stain magenta, and lambda targets will stain black. The typical positive staining pattern for B-cells is a partial to full ring of punctate cytoplasmic staining, while plasma cells typically exhibit complete filling of the cytoplasm due to abundant mRNA.

The staining pattern is interpreted as a ratio of kappa to lambda expressing cells for the determination of restriction status. The normal immune response typically produces a kappa-heavy polyclonal population of approximately 2-3:1 kappa to lambda. For VENTANA K/L Probe Cocktail, a ratio greater than 4:1 is interpreted as kappa restricted, and a ratio lower than 1:2 is interpreted as lambda restricted (see Table 4 and Figure 4).

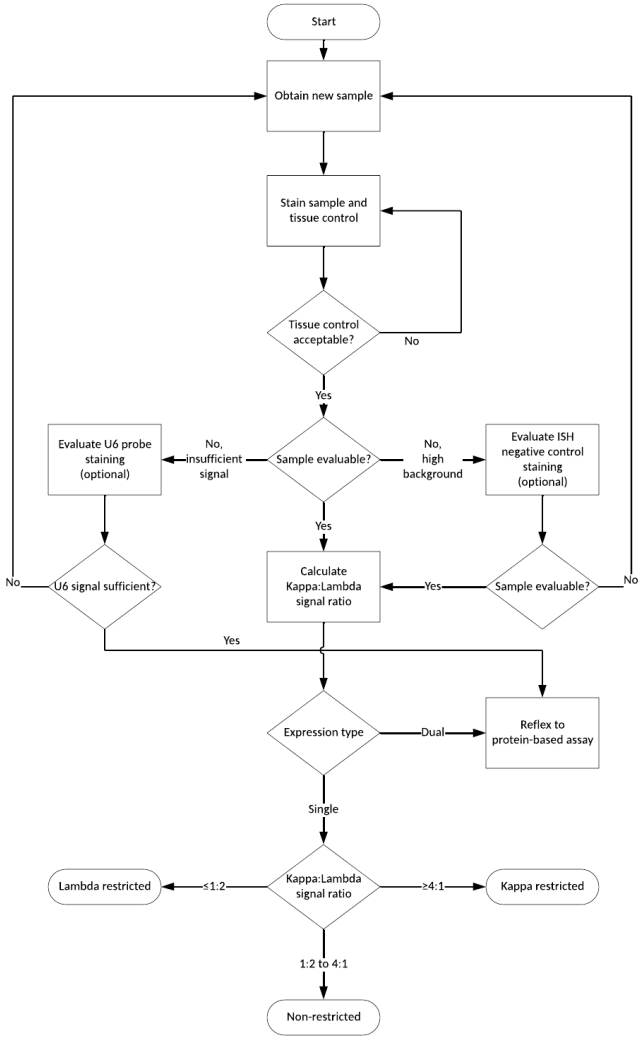
The transcript for IGLL5 is 100% homologous with the lambda light chain sequence. Due to this homology, VENTANA K/L Probe Cocktail may stain IGLL5 mRNA. IGLL5 expression is predominantly nuclear, and its signal is visualized as black, punctate dots. Based on internal testing, prominent IGLL5 signal is observed in approximately 15% of cases and should not be interpreted in determination of restriction status.

Based on internal testing, approximately 5% of lymphoma and myeloma cases exhibit the presence of both kappa and lambda mRNA in the cytoplasm of the same cells. Clonality cannot be determined by ISH for these cases, and reflex testing to a protein-based assay should be done. Co-expression of signal is not observed in non-restricted tissues.

Table 4. Scoring criteria for the determination of restriction status.

Clinical Status	Kappa:Lambda Ratio
Kappa restricted	Greater than or equal to 4:1
Non-restricted	Less than 4:1 and greater than 1:2
Lambda restricted	Less than or equal to 1:2

Figure 4. Decision tree for the interpretation of VENTANA K/L Probe Cocktail staining in controls and patient cases.



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 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. 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. Not all fixatives may be compatible with the VENTANA K/L Probe Cocktail assay. Ventana recommends fixation with 10% NBF for 6-72 hours. Refer to Table 5 for fixatives that have been tested. Fixation time should be validated prior to use.
2. VENTANA K/L Probe Cocktail 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 probe. Refer to Table 6 for specific reagents that have been tested. Decalcification time should be validated prior to use.
3. The VENTANA K/L Probe Cocktail is not intended for use in the diagnosis of B-cell lymphomas or reactive B-cell processes in bone marrow cores due to the degradation of mRNA by many commonly used decal solutions.
4. Based on internal testing, approximately 5% of lymphoma and myeloma cases exhibit the presence of both kappa and lambda mRNA in the cytoplasm of the same cells. Clonality cannot be determined by ISH for these cases, and reflex testing to a protein-based assay should be done.
5. The VENTANA K/L Probe Cocktail assay was developed to be used with tissues cut to 4µm thickness. Sections thinner/thicker than this may experience inappropriate staining and/or tissue loss.
6. Tissues should be stained within 60 days of sectioning. Loss of staining may be observed in tissues stained after 60 days.
7. Not all mounting media may be compatible with the chromogens used in the VENTANA K/L Probe Cocktail assay. Specifically, oxidation, fading, and/or disappearance of the silver signal may occur with certain brands of mounting media. Refer to Table 7 for mounting media that have been tested.
8. Stained slides should be stored in the dark when not in use to prevent fading or hue change of the magenta and silver chromogens.
9. 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.
10. Due to variations in specimen processing or pathologist preference, it may be necessary to select the “Short Pre-Treatment” option in the protocol. This change must be validated by the user. Users who deviate from the recommended test procedure are responsible for interpretation of patient results under these circumstances.
11. This probe 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.
12. All assays might not be registered on every instrument. Please contact your local support representative for more information.

Table 5. 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 6. 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 7. 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 performance of the VENTANA K/L Probe Cocktail was evaluated through clinical and analytical studies. All staining was performed using the VENTANA K/L Probe Cocktail protocol as described in Table 2 on a BenchMark IHC/ISH instrument unless otherwise specified.

CLINICAL PERFORMANCE

Method Comparison Study of VENTANA Kappa/Lambda Dual ISH mRNA Probe Cocktail Assay Performance, as Used on the BenchMark ULTRA Instrument with VENTANA Silver ISH BF and Magenta ISH DIG Detection Kits, Relative to Flow Cytometry in the Determination of Kappa/Lambda Restriction Status

An external multi-site study was conducted to evaluate concordance of the VENTANA K/L Probe Cocktail assay to flow cytometry for determination of light chain restriction status in lymphoid and bone marrow specimens. Three clinical sites screened a total of 1019 cases for potential enrollment in the study. The clinical sites stained 869 of these cases with VENTANA K/L Probe Cocktail on a BenchMark ULTRA instrument, and 811 of the 869 stained cases had acceptable H&E and were evaluated for K/L staining by the screening pathologist. A total of 742 cases that met enrollment criteria were enrolled into the study and up to 4 pathologists from the sites each evaluated all enrolled cases.

Performance of the VENTANA K/L Dual ISH assay was assessed as percent agreement between the ISH assay and historical flow results for each restriction category (kappa restricted, lambda restricted, or non-restricted) and as an overall percent agreement (OPA) for all cases.

Table 8. Restriction status agreement between VENTANA K/L Probe Cocktail and flow cytometry for all readers pooled.

VENTANA K/L Dual ISH Result	Flow Cytometry Result			
	Kappa Restricted	Lambda Restricted	Non-restricted	Total
Kappa Restricted	824	16	23	863
Lambda Restricted	16	742	18	776
Non-restricted	76	37	810	923
Total	916	795	851	2562

Table 9. Pooled agreement of K/L restriction status between the VENTANA K/L Dual ISH Assay and Flow Cytometry (all evaluable cases).

Restriction Category	Agreement % (n/N)	95% CI
Kappa Restricted	90.0% (824/916)	86.7% – 93.2%
Lambda Restricted	93.3% (742/795)	90.1% – 96.2%
Non-restricted	95.2% (810/851)	93.3% – 96.8%
Overall	92.7% (2376/2562)	91.1% – 94.2%

ANALYTICAL PERFORMANCE

Sensitivity and Specificity

For sensitivity and specificity testing, VENTANA K/L Probe Cocktail was used to stain a panel of normal and neoplastic FFPE tissues. Stained slides were evaluated as positive/negative for kappa and lambda signal by a pathologist. No unexpected staining was observed for any of the stained cases.

Table 10. VENTANA K/L Probe Cocktail staining in normal tissues. Tissues marked with an asterisk exhibited scattered normal B-lymphocyte staining.

Pathology	# Positive / Total Cases
Brain	0/1
Cerebrum	0/3
Cerebellum	0/4
Adrenal gland*	0/4
Ovary*	0/4
Pancreas*	0/4
Lymph node	15/15
Parathyroid gland	0/3
Pituitary gland	0/3
Testis*	0/4
Thyroid	0/4
Breast*	0/5
Spleen*	0/4
Tonsil	5/5
Skeletal muscle*	0/3
Peripheral nerve	0/4

Pathology	# Positive / Total Cases
Bladder	0/4
Thymus*	0/4
Bone marrow	5/5
Lung*	0/5
Heart	0/4
Esophagus*	0/4
Stomach*	0/4
Small intestine*	0/4
Colon*	0/6
Liver*	0/4
Salivary gland*	0/4
Kidney*	0/5
Prostate*	0/4
Cervix	0/6
Skin	0/4
Mesothelial	0/4
Rectum	0/1
Placenta	0/3
Uterus*	0/4

Table 11. VENTANA K/L Probe Cocktail staining in neoplastic tissues.

Pathology	# Positive / Total Cases
Astrocytoma (Brain)	0/1
Meningioma, (Brain)	0/3
Adenocarcinoma (Head and neck)	0/1
Melanoma (Nasal cavity)	0/1
Nasopharyngeal carcinoma, (Nasopharynx)	0/1
Squamous cell carcinoma (Tongue)	0/1
Adenoma (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	0/1
Colonic signet ring cell carcinoma, metastatic (Ovary)	0/1
Adenocarcinoma (Ovary)	0/2
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Adenoma (Thyroid)	0/3
Follicular carcinoma (Thyroid)	0/1
Follicular papillary adenocarcinoma (Thyroid)	0/1
Invasive ductal carcinoma (Breast)	0/3
Fibroadenoma (Breast)	0/2
Osteosarcoma (Bone)	0/1
Chondrosarcoma (Bone)	0/1
Adenocarcinoma (Lung)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Gastrointestinal carcinoma, metastatic (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/2
Adenocarcinoma (Stomach)	0/3
Adenoma (Small intestine)	0/1
Adenocarcinoma (Small intestine)	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	0/3
Colonic adenocarcinoma, metastatic (Liver)	0/1
Hepatocellular carcinoma (Liver)	0/4
Pleomorphic adenoma (Parotid salivary gland)	0/1
Adenoid cystic carcinoma (Salivary gland)	0/1
Clear cell carcinoma (Kidney)	0/2
Adenocarcinoma (Prostate)	0/2
Adenocarcinoma (Uterus)	0/2
Squamous cell carcinoma (Cervix)	0/2
Squamous cell carcinoma (Skin)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/2
T-Cell lymphoblastic lymphoma	0/1
T cell lymphoma, Mycosis fungoides	0/1
T cell lymphoma, Lennert lymphoma	0/2
T cell lymphoma, enteropathy-associated	0/5
T cell lymphoma, angioimmunoblastic	0/6
T cell lymphoma, NOS	0/20
NK/T-cell lymphoma, nasal type	0/5
NK/T-cell lymphoma (Testis)	0/1
Hodgkin lymphoma	0/1
B-cell lymphoma, NOS	1/1
Follicular lymphoma	7/7
Mantle cell lymphoma	4/4

Pathology	# Positive / Total Cases
Diffuse large B-cell lymphoma	11/11
Plasma cell neoplasms	4/4
Marginal zone lymphoma	3/3
Chronic lymphocytic leukemia/small lymphocytic lymphoma	5/5
Urothelial carcinoma (Bladder)	0/2
Squamous cell carcinoma, metastatic (Lymph node)	0/1
Adenocarcinoma (Rectum)	0/3

Within-Run Repeatability, Between-Day Intermediate Precision, and Between-Instrument Intermediate Precision

Repeatability and precision of the VENTANA K/L Probe Cocktail was assessed by staining a panel of 26 cases on multiple BenchMark ULTRA instruments. The panel of cases contained both normal and neoplastic specimens and represented a range of restriction statuses and mRNA expression levels. Randomized slides were evaluated for restriction status by a pathologist blinded to case diagnoses.

For within-run repeatability, duplicate sections from each specimen were stained on 7 independent instrument runs. Repeatability was calculated by OPA for all slides as determined by individual case level modal restriction status.

For between-day intermediate precision, duplicate sections from each specimen were stained on a single BenchMark ULTRA instrument across 5 non-consecutive days. Intermediate precision was calculated by OPA for all slides as determined by individual case level modal restriction status.

For between-instrument intermediate precision, duplicate sections from each specimen were stained on 3 different BenchMark ULTRA instruments. Intermediate precision was calculated by OPA for all slides as determined by individual case level modal restriction status.

Table 12. Results for repeatability and intermediate precision testing on the BenchMark ULTRA platform.

Study	n/N	OPA	95% CI
Within-Run Repeatability	178/178	100%	97.9% - 100.0%
Between-Day Intermediate Precision	256/256	100%	98.5% - 100.0%
Between-Instrument Intermediate Precision	156/156	100%	97.6% - 100.0%

BenchMark Platform Concordance

Platform concordance of the VENTANA K/L Probe Cocktail assay was assessed by staining a panel of 59 cases on both the BenchMark ULTRA and ULTRA PLUS instruments. The panel of cases contained both normal and neoplastic specimens and represented a range of restriction statuses and mRNA expression levels. Randomized slides were evaluated for restriction status by a pathologist blinded to case diagnoses.

Table 13. Results for BenchMark ULTRA and ULTRA PLUS platform concordance.

Study	n/N	OPA	95% CI
Platform Concordance	59/59	100%	93.9% - 100.0%

Reader Precision

Within-reader and between-reader precision of the VENTANA K/L Probe Cocktail was assessed by comparing restriction status evaluations from 3 pathologists for a panel of 60 stained slides. The panel of cases contained both normal and neoplastic specimens and represented a range of restriction statuses and mRNA expression levels. For the study, randomized slides were evaluated for restriction status by 3 pathologists blinded to case diagnoses. Following a 2-week washout period, the stained slides were re-randomized and re-evaluated for restriction status by the same 3 pathologists.

For within-reader precision, round 1 evaluations were compared to round 2 evaluations for each individual pathologist's reads. Within-reader precision was calculated by OPA for the pooled concordance data for all 3 pathologists.

For between-reader precision, the evaluations from each pathologist were compared to the evaluations of the other 2 readers. Between-reader precision was calculated by OPA for all evaluations for all 3 pathologists as determined by case level modal restriction status.

Table 14. Results for reader precision testing.

Study	n/N	OPA %	95% CI
Within-Reader Precision	180/180	100%	97.9% - 100.0%
Between-Reader Precision	352/360	97.8%	94.4% - 100.0%

Lot-to-Lot Precision

Lot-to-lot precision of the VENTANA K/L Probe Cocktail was assessed by staining a panel of 26 cases with 3 production lots of the probe cocktail. The panel of cases contained both normal and neoplastic specimens and represented a range of restriction statuses and mRNA expression levels. Randomized slides were evaluated for restriction status by a pathologist blinded to case diagnoses.

Lot-to-lot precision was calculated by OPA for all slides as determined by individual case level modal restriction status.

Table 15. Results for lot-to-lot precision testing.

Study	n/N	OPA %	95% CI
Lot-to-Lot Precision	78/78	100%	95.3% - 100.0%

Inter-Laboratory Reproducibility

An inter-laboratory reproducibility study was conducted to evaluate reproducibility of the VENTANA K/L Probe Cocktail assay for determining restriction status. For this study, 3 external laboratories each stained a panel of 28 cases on 5 non-consecutive days over a 21 day period on a BenchMark ULTRA. The panel of cases contained both normal and neoplastic specimens (8 kappa restricted, 8 lambda restricted, and 12 non-restricted cases) and represented a range of restriction statuses and mRNA expression levels.

Randomized slides were independently evaluated for restriction status by 2 pathologists per site (6 total) blinded to case diagnoses. VENTANA K/L Probe Cocktail stained slides were presented for interpretation together with case-matched H&E and ISH Negative Control stained slides.

Reproducibility was assessed as percent agreement between individual restriction evaluations for each case and the modal case restriction status for that case for each restriction category (kappa restricted, lambda restricted, or non-restricted) and as an overall percent agreement for all cases (Table 16).

Additionally, the average pairwise agreement rate for each restriction status between-site, between-readers, and between-days were reported (Table 17).

Table 16. Results for agreement between individual evaluations and modal case restriction status (KRPA = kappa restricted percent agreement, LRPA = lambda restricted percent agreement, NRPA = non-restricted percent agreement)

Restriction Category	Agreement % (n/N)	95% CI
KRPA	98.8% (237/240)	97.5% – 99.6%
LRPA	99.6% (239/240)	98.8% – 100.0%
NRPA	97.5% (351/360)	96.1% – 99.0%
OPA	98.5% (827/840)	97.7% – 99.2%

Table 17. Results for overall between-site, between-reader, and between-day pairwise agreements for restriction status.

Pairwise Factor	Restriction Category	Agreement	95% CI
Between Sites	Kappa Restricted	95.6%	89.3% – 98.1%
	Lambda Restricted	98.8%	96.2% – 100.0%
	Non-restricted	96.6%	94.2% – 98.4%
	OPA	96.9%	95.2% – 98.6%
Between Readers	Kappa Restricted	95.5%	89.2% – 98.1%
	Lambda Restricted	98.8%	96.2% – 100.0%
	Non-restricted	96.6%	94.2% – 98.4%
	OPA	96.9%	95.1% – 98.6%
Between Days	Kappa Restricted	95.5%	89.2% – 98.1%
	Lambda Restricted	98.8%	96.2% – 100.0%
	Non-restricted	96.6%	94.2% – 98.4%
	OPA	96.9%	95.1% – 98.6%

TROUBLESHOOTING

Table 18. Troubleshooting solutions.

Issue	Solution
Absent/weak or inappropriate background in the pre-qualified on-slide tonsil control tissue	1. Check that the slide has the proper barcode label and that the correct probe was selected in the protocol. If the protocol uses "Short Pre-Treatment", ensure that the tonsil was qualified with a protocol using the same condition. 2. Ensure that the section was cut at 4 µm and a compatible mounting medium was used. 3. 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. 4. If the controls on other VENTANA K/L Probe Cocktail-stained slides run at the same time stained appropriately, an unknown slide-specific failure may have occurred. Prepare a new slide and restain. 5. Contact your local representative for support if other VENTANA K/L Probe Cocktail-stained slides run at the same time also produced inadequate staining, and no obvious source of failure can be identified (e.g. the staining properties of the qualified tonsil block have changed as the block is cut through).
Absent/weak staining in the patient specimen with appropriate staining in the pre-qualified on-slide tonsil control	1. Ensure that the section was cut at 4 µm and that compatible fixation and/or decalcification methods were used to process the specimen. Inappropriate decalcification processes may destroy target RNA. 2. If the slide was stained using a protocol with the "Short Pre-Treatment" selection, re-staining with normal pre-treatment may produce a darker signal. 3. Stain a slide from the patient specimen with the VENTANA U6 BF probe to evaluate for mRNA integrity. If loss of mRNA is observed, a new patient specimen may be required. 4. If mRNA integrity does not appear to be impacted, Kappa and/or Lambda mRNA expression may be absent or below the threshold of detection with the assay.
Inappropriate background in the patient specimen with appropriate staining in the pre-qualified on-slide tonsil control	1. Ensure that the section was cut at 4 µm and compatible fixation and/or decalcification methods were used to process the specimen. 2. If the slide was stained using the recommended protocol, re-staining with "Short Pre-Treatment" selected may reduce background signal. 3. Stain a slide from the patient specimen with ISH Negative Control to evaluate for detection kit associated background. Subtract detection background from the VENTANA K/L Probe Cocktail stained slide to interpret the restriction status. 4. If background still interferes with the ability to determine restriction status, a new specimen may be required.
IGLL5 signal interferes with Kappa/Lambda signal evaluation	1. If the slide was stained using the recommended protocol, re-staining with "Short Pre-Treatment" selected may reduce the IGLL5 signal. 2. If IGLL5 signal still interferes with the ability to interpret kappa/lambda signal, reflex testing to a protein-based assay may be required to evaluate restriction status.
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
UDI	Unique Device Identifier
	Indicates the entity importing the medical device into the European Union

INTELLECTUAL PROPERTY

CONTACT INFORMATION

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

www.roche.com

CE 0123