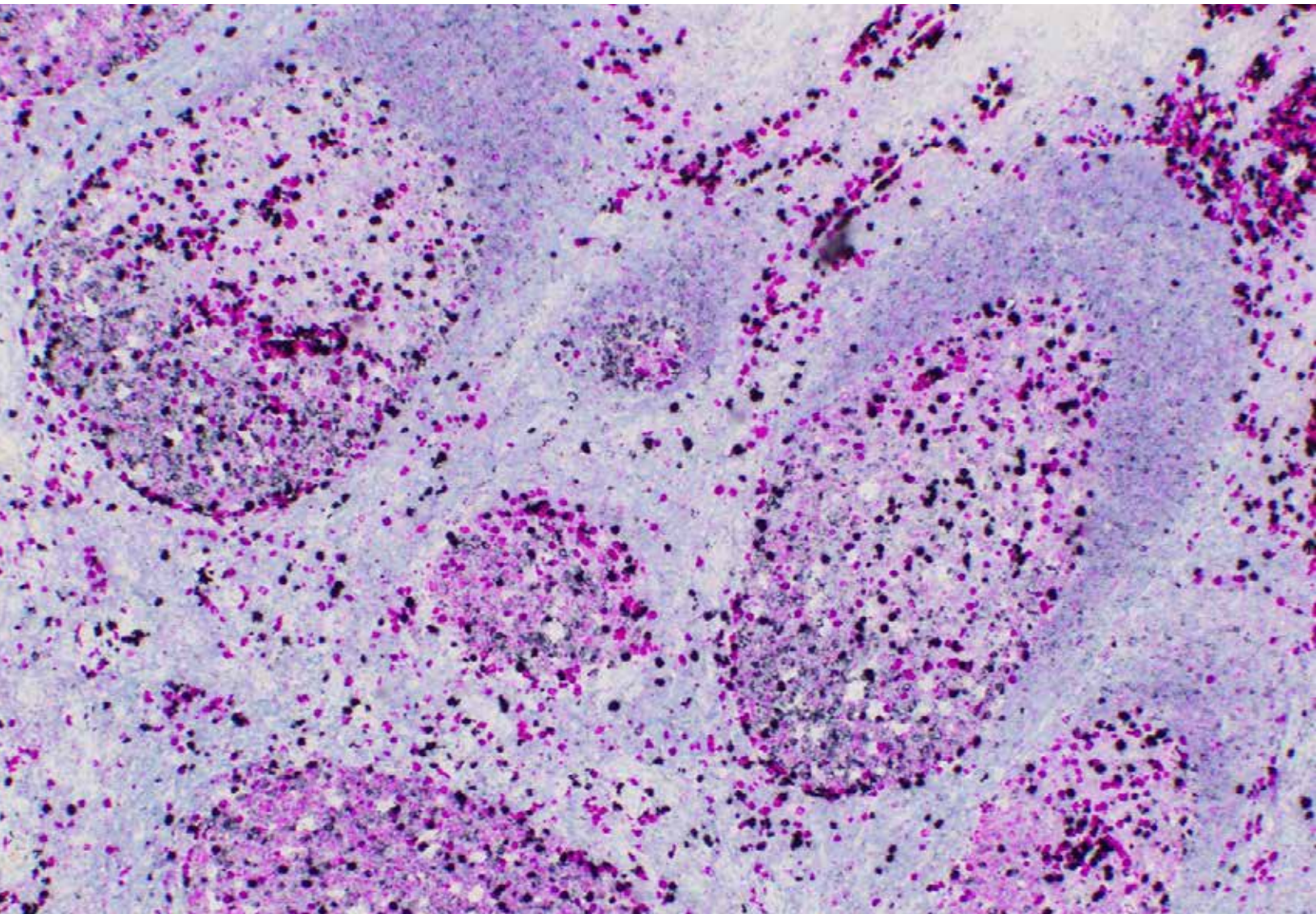
Interpretation Guide for VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail for B-cell Lymphomas and Plasma Cell Neoplasms



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Introduction

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.

The 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.

The VENTANA K/L Probe Cocktail is a mix of benzofurazan (BF) and digoxigenin (DIG) 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. Lambda target is visualized in black with the VENTANA Silver ISH BF Detection Kit, and kappa target is visualized in magenta with the VENTANA Magenta ISH DIG Detection Kit. Restriction status is determined by assessing the ratio of kappa to lambda signal.

Intended Use

Intended Use of Product

Refer to the corresponding VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail method sheet (102202117EN) for detailed use of this product.

Note: Use of this diagnostic with indicated therapies may not be approved in all countries. Please consult your local Roche representative for local approvals.

Purpose of Interpretation Guide

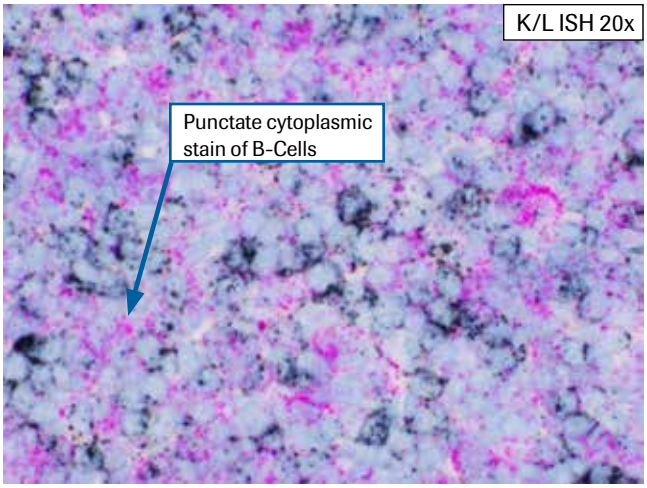
This guide is intended to:

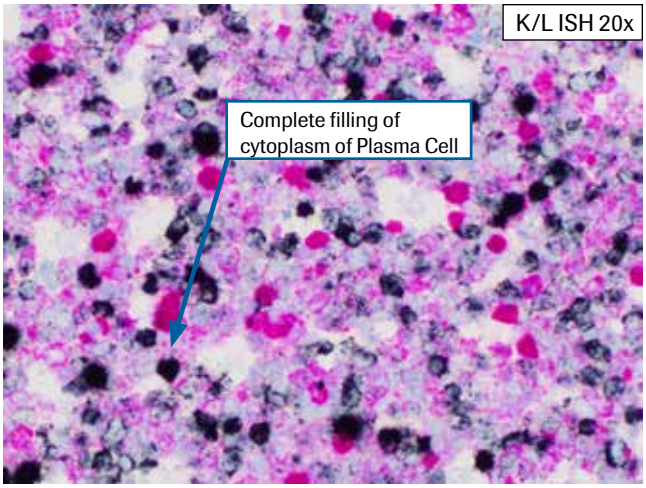
- Provide pathologists with a tool to facilitate clinical evaluation of formalin-fixed, paraffin-embedded (FFPE) human bone marrow and lymphoid tissue sections stained with VENTANA K/L Probe Cocktail in accordance with the proposed product labeling.
- Provide photographic images that illustrate the patterns and intensities of staining that may result from staining of bone marrow or lymphoid tissues with VENTANA K/L Probe Cocktail.
- Provide examples images of challenging cases to provide guidance in their evaluation.
- Provide guidance in using tissue controls or integrity assays that may be stained with VENTANA K/L Probe Cocktail.

Clinical Evaluation

Evaluating Staining Patterns

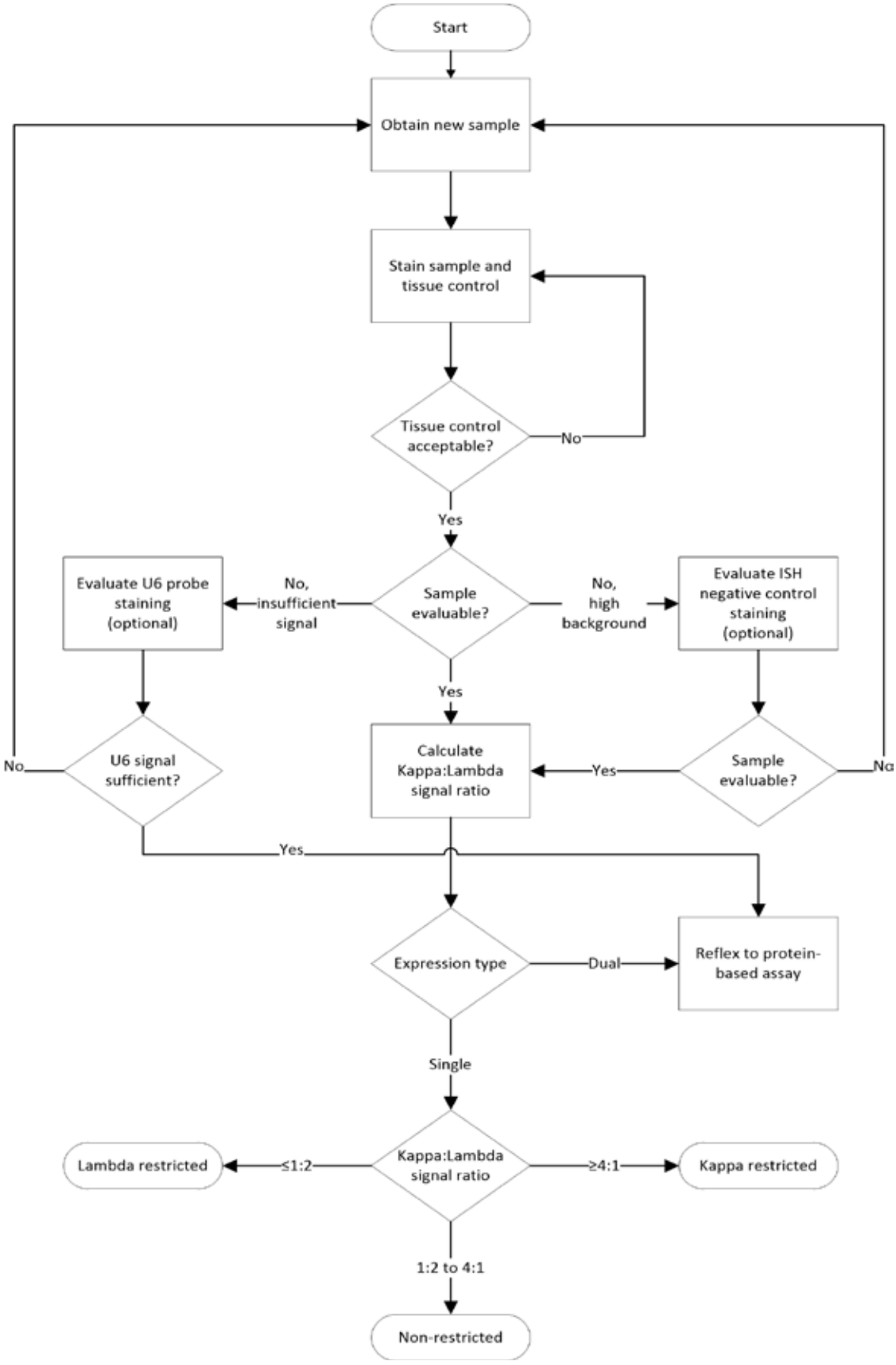
With the VENTANA K/L Probe Cocktail assay, kappa target will stain magenta, and lambda target will stain black. The typical positive staining pattern for B-cells is a partial to full ring of punctate cytoplasmic staining, while plasma cells typically exhibit complete filling of the cytoplasm due to abundant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





Staining Patterns: The images above demonstrate the staining pattern of the VENTANA K/L Probe Cocktail. The image on the left shows B-cells with a partial or full ring of punctate cytoplasmic staining, while the image on the right shows the complete filling of the cytoplasm in the plasma cells.

Specimen Flow



Scoring Criteria

To determine restriction status, the percent of cells expressing kappa light chain mRNA is compared to the percent of cells expressing lambda mRNA. The normal immune response typically produces a greater kappa polyclonal population with a ratio of approximately 2-3:1 kappa to lambda. Restricted populations will produce an excess of one light chain over the other. For the 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 1**).

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. Negative control staining is not required for interpretation of restriction status.

Table 1: 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

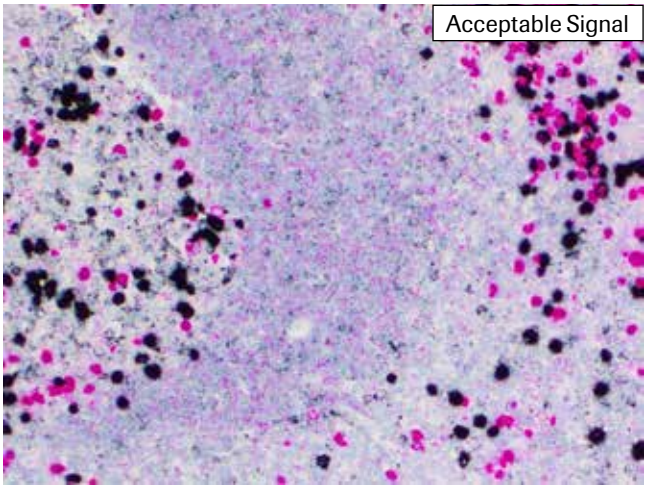
On Slide Control

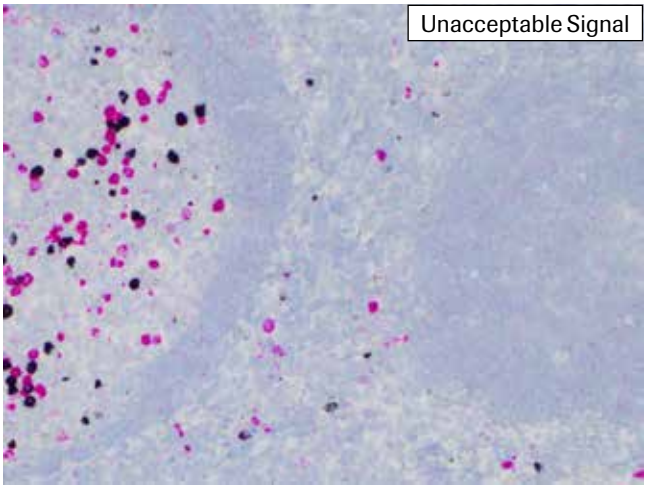
It is recommended that a laboratory-specific tonsil control tissue should be included on each patient slide to ensure that the assay is performing as expected. Normal tonsil can serve as an on slide control tissue, showing both positive staining in B-cells and plasma cells and negative staining in T cells, stroma and squamous epithelium.

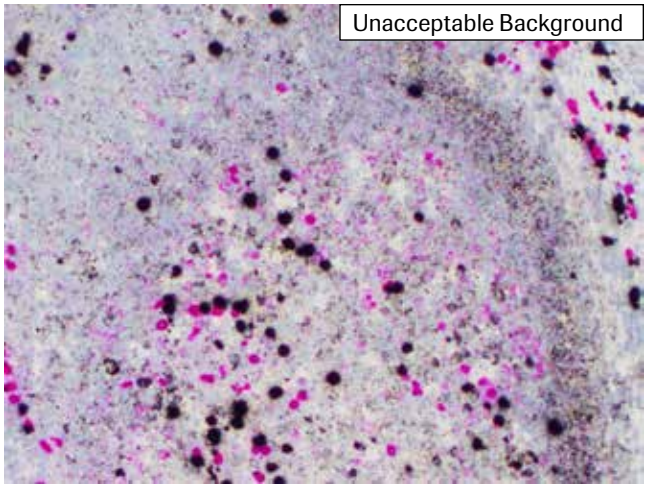
On slide tonsils should be qualified and demonstrate staining of both kappa and lambda mRNA in the majority of the mantle zones that is clearly visualized at 10x. Qualified tonsils should also exhibit minimal background staining in the stroma and non-B-cell areas. Once qualified, the tonsil can serve as an on slide control, ensuring proper functioning of the assay. Markedly diminished signal or excessive background indicates that an error may have occurred on that slide.

Table 2: Scoring Criteria for determination of unacceptable tonsil staining

Staining Element	Acceptable Staining	Unacceptable Staining
Positive staining	Punctate cytoplasmic dot staining of almost all B-cells in the mantle zone, clearly visible at 10X, with physiologic K/L ratio (2:1-3:1)	Markedly diminished staining in the majority of B-cells in the mantle zone, requiring 20X for visualization
Negative staining	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







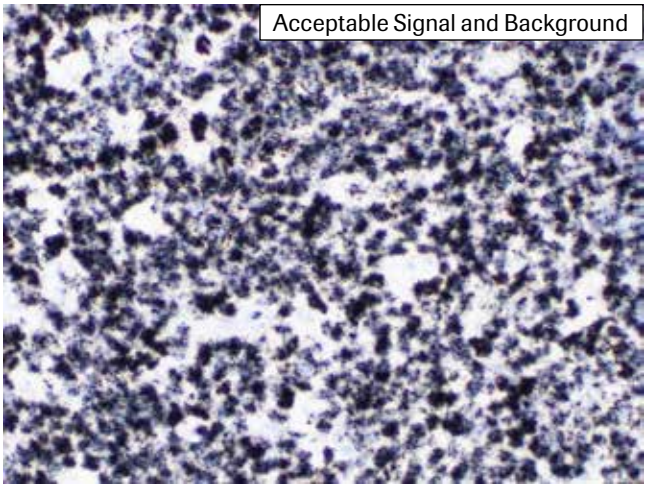
mRNA Integrity Assessment

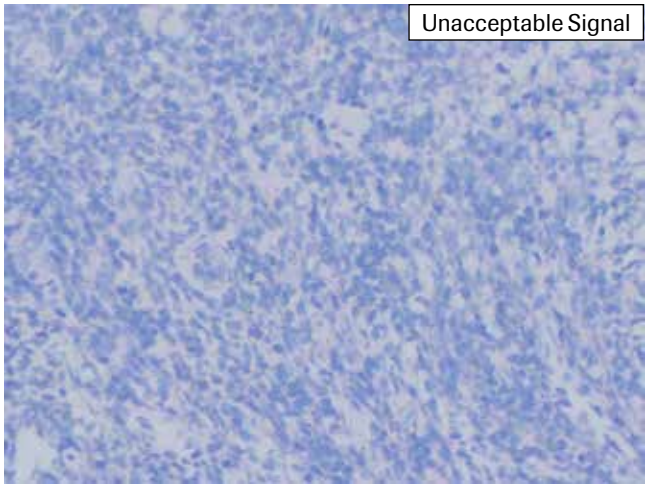
The VENTANA K/L Probe Cocktail assay may have reduced performance on tissues where mRNA integrity has been impacted. Because RNA is susceptible to degradation, the ubiquitously expressed U6 snRNA transcript is commonly used as a surrogate to assess RNA integrity. The VENTANA U6 BF Probe (Cat. No. 760-7062 / 08773866001) is used with the VENTANA Silver ISH BF Detection Kit 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.

Table 3: Scoring criteria for determination of acceptable/unacceptable U6 mRNA probe staining

Staining Element	Acceptable Staining	Unacceptable Staining
Positive staining	Nuclear signal is clearly visible in the majority of tumor cell nuclei	Nuclear signal is not clearly visible in the majority of tumor cell nuclei
Negative staining	Absent or low scattered and randomly distributed staining in the cytoplasm of cells	Excessive non-specific background staining of cytoplasm of cells preventing enumeration of U6 status

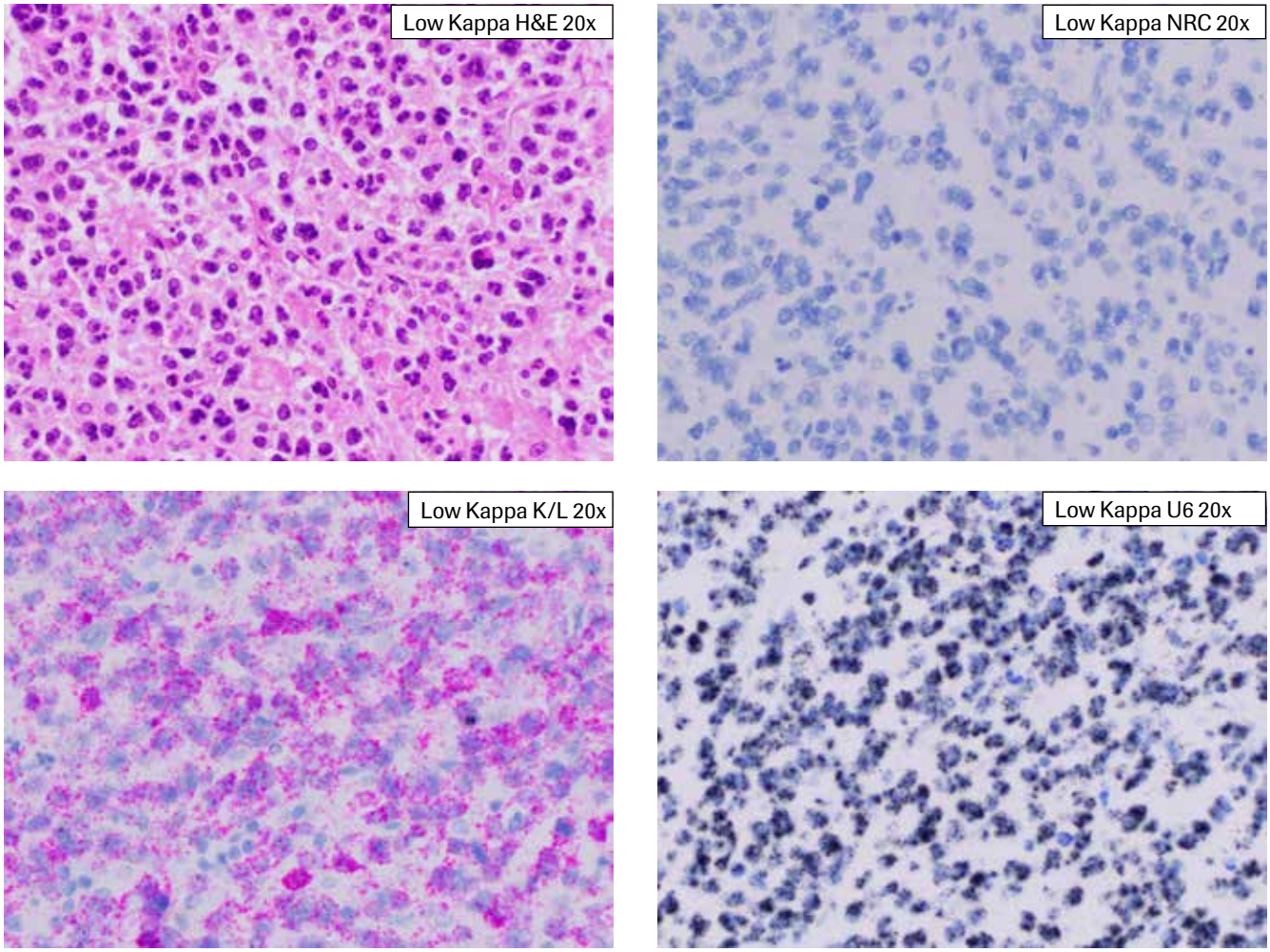




Restriction Status

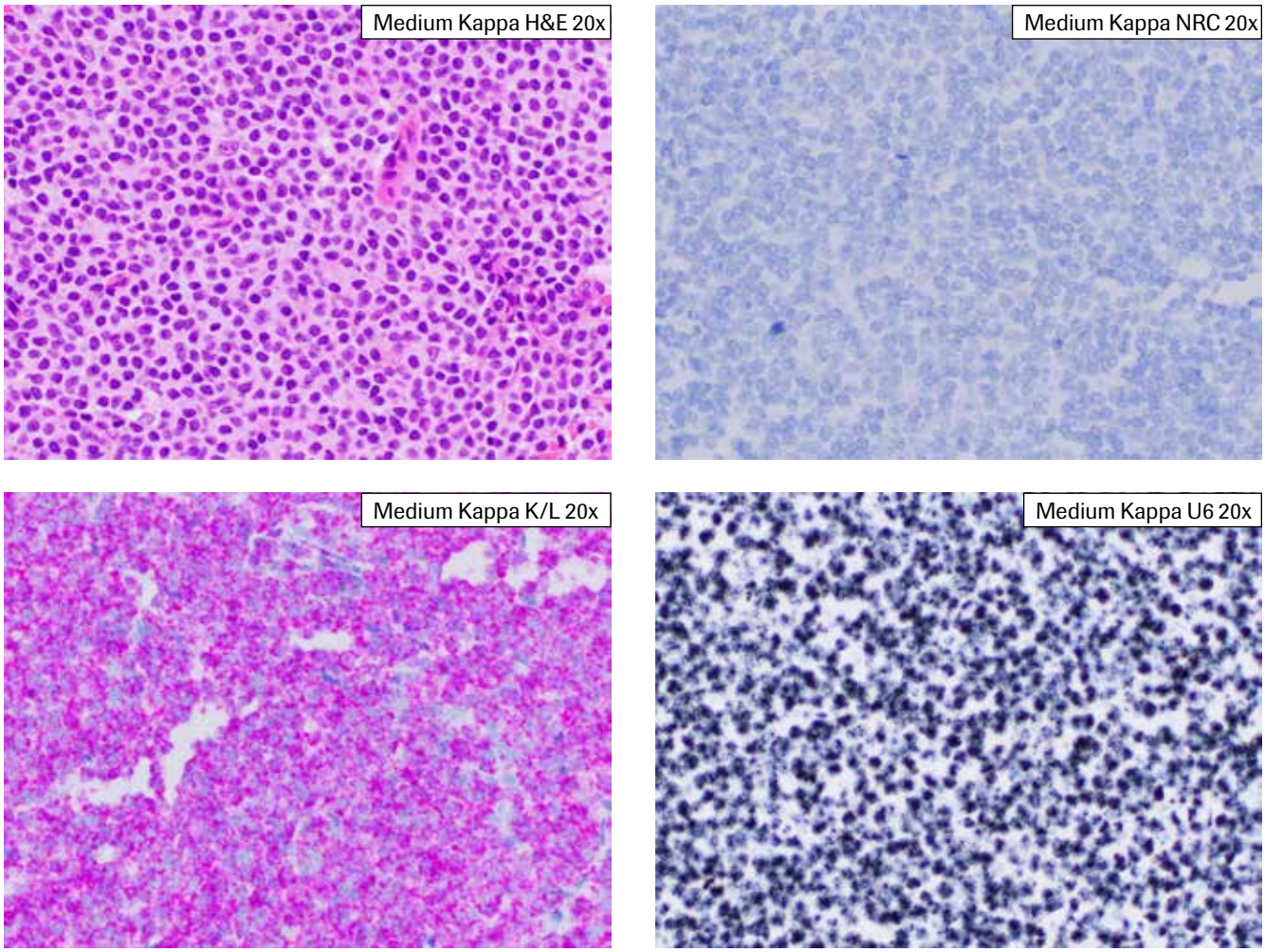
Kappa Restricted: Low Expressing

Cases with a kappa (magenta): lambda (black) ratio greater than or equal to 4:1 are considered kappa restricted. Kappa expressing cells themselves can have a wide range of expression from several magenta dots per cell, to the entire of a cell filled with magenta signal.



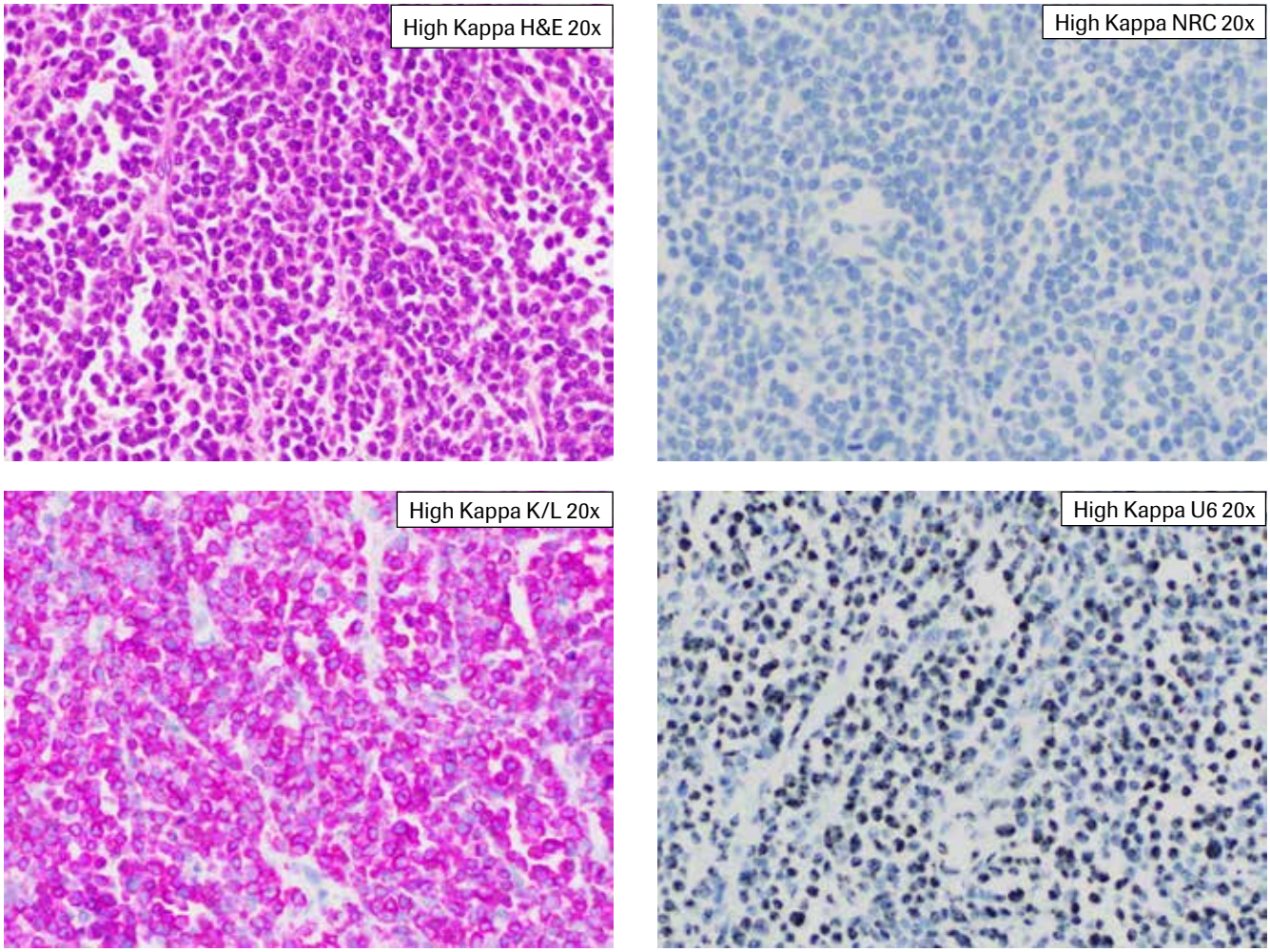
Kappa Restricted – Low Expressing: The images above show a diffuse large B-Cell lymphoma (DLBCL) stained with Hematoxylin and Eosin (H&E), Negative Reagent Control (NRC), VENTANA K/L Probe Cocktail (K/L), and VENTANA U6 BF Probe (U6).

Kappa Restricted: Medium Expressing



Kappa Restricted – Medium Expressing: The images above show a marginal zone lymphoma (MZL) stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe.

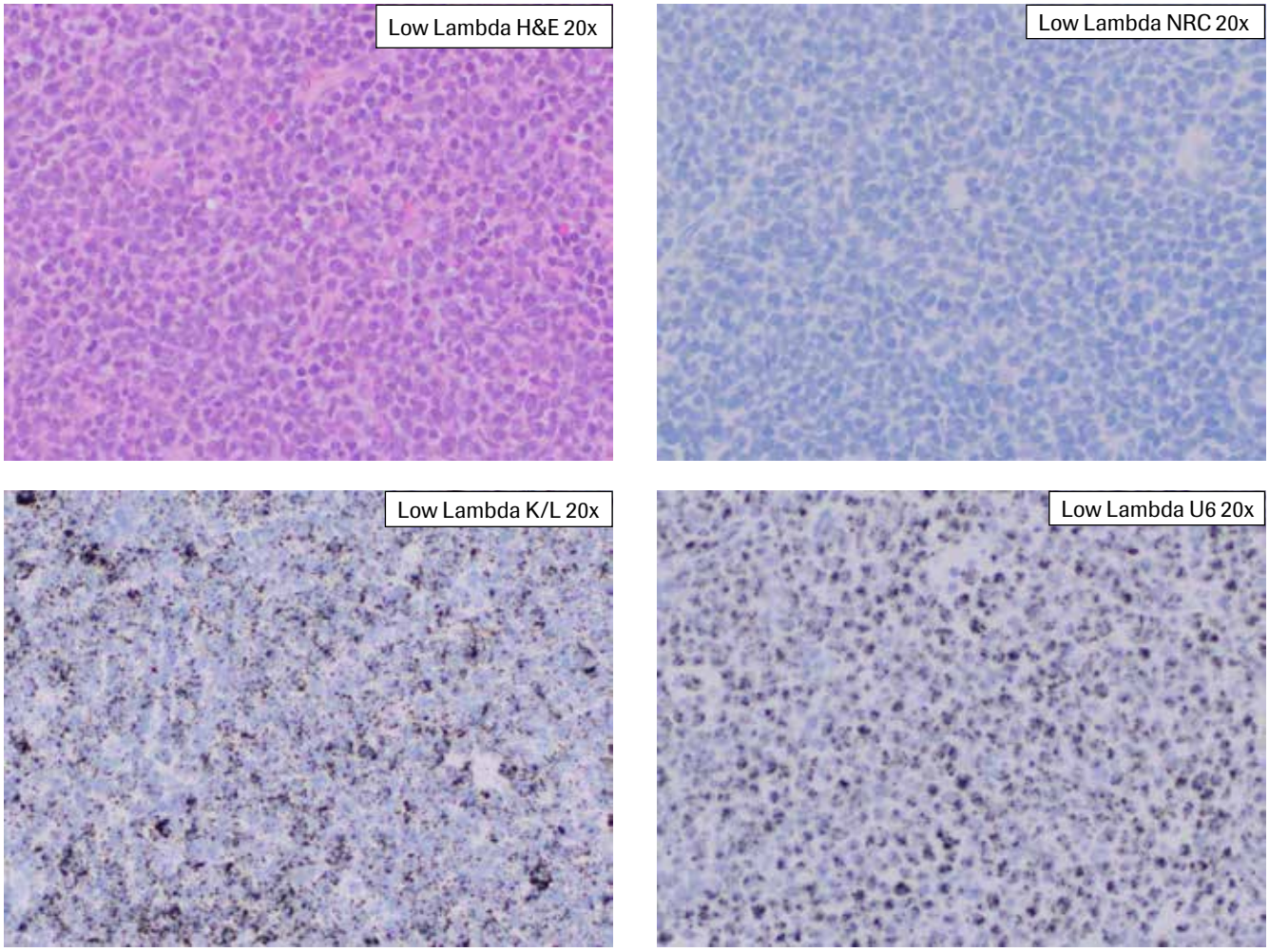
Kappa Restricted: High Expressing



Kappa Restricted – High Expressing: The images above show a plasmacytoma stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe.

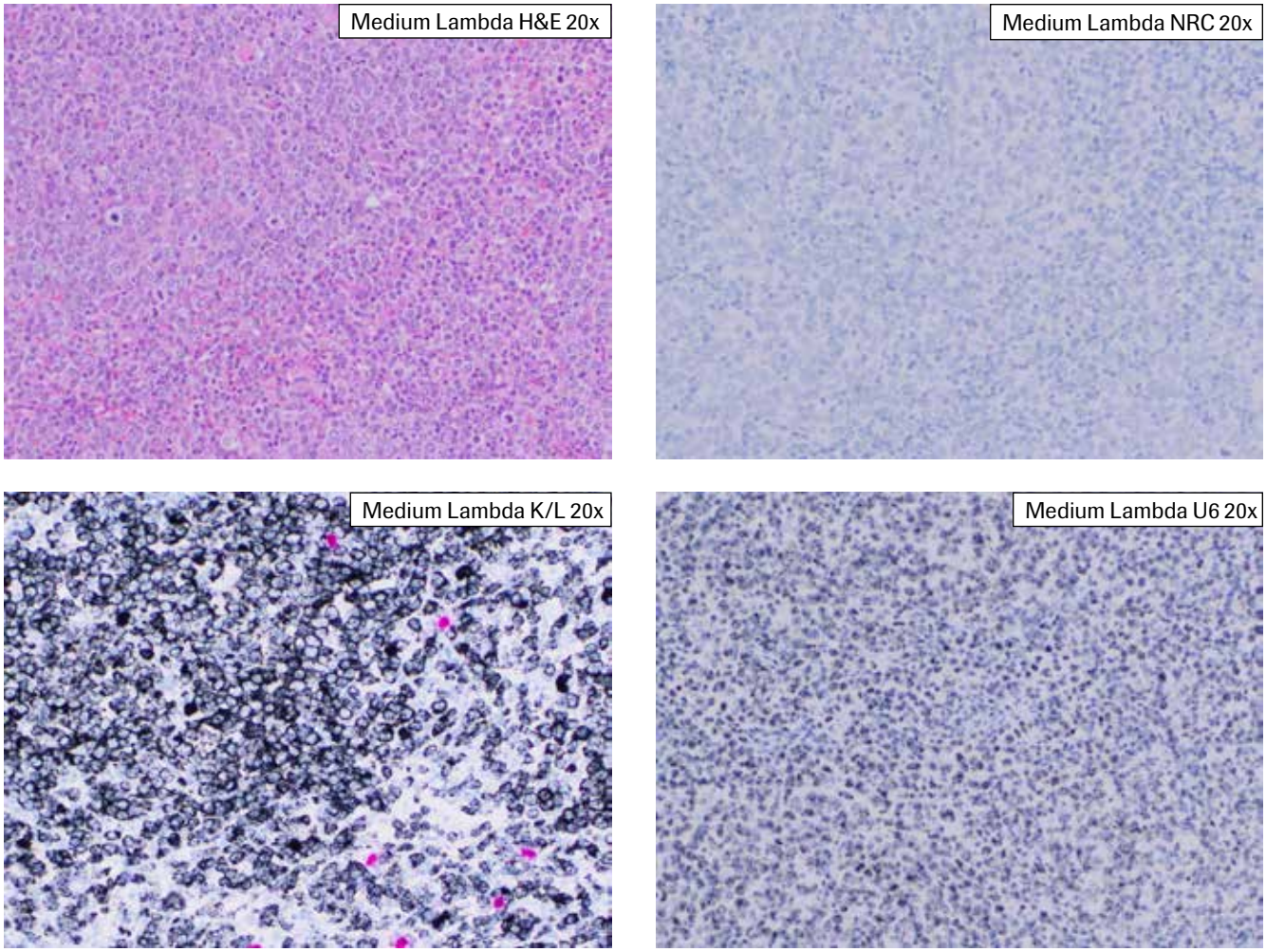
Lambda Restricted: Low Expressing

Cases with a kappa (magenta): lambda (black) ratio less than or equal to 1:2 are considered lambda restricted. Lambda expressing cells can have a wide range of expression from several SISH (Silver *In Situ* Hybridization) dots per cell to the entire cell filled in with SISH signal. Regardless of expression level, any cells that meet this description are lambda expressing cells.



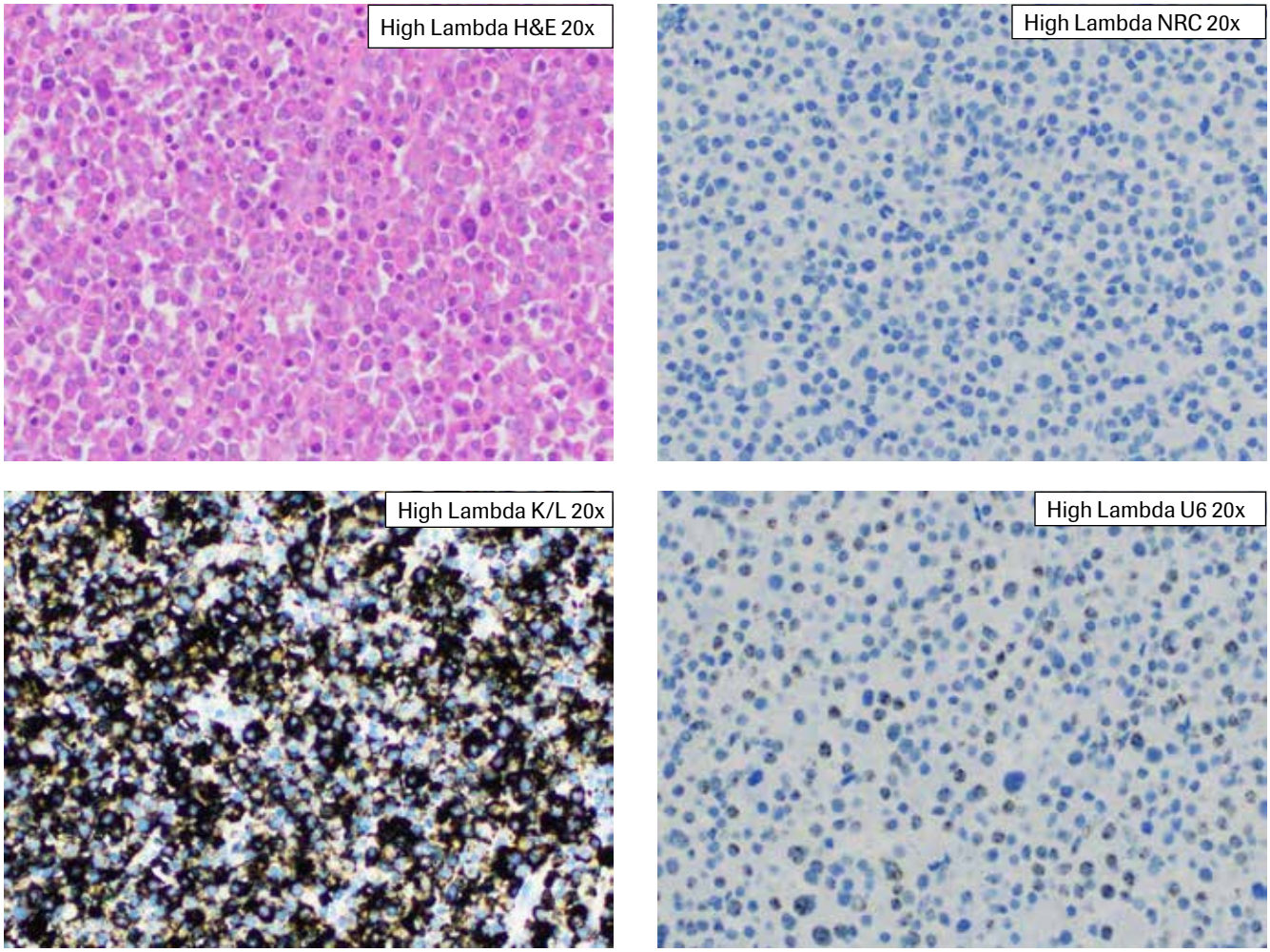
Lambda Restricted – Low Expressing: The images above show a follicular lymphoma stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe.

Lambda Restricted: Medium Expressing



Lambda Restricted – Medium Expressing: The images above show a follicular lymphoma stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe.

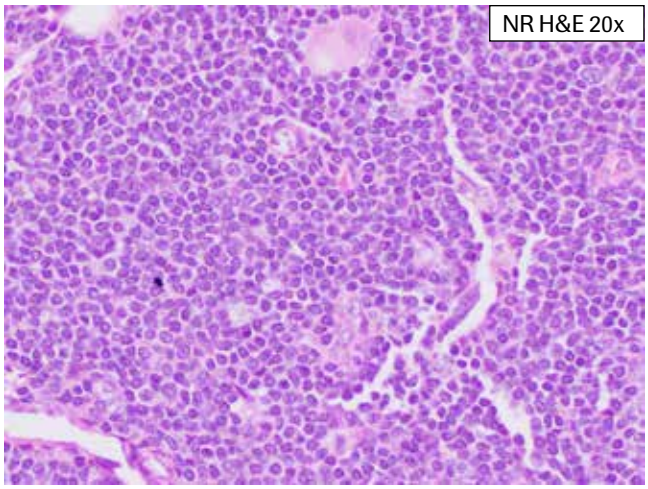
Lambda Restricted: High Expressing

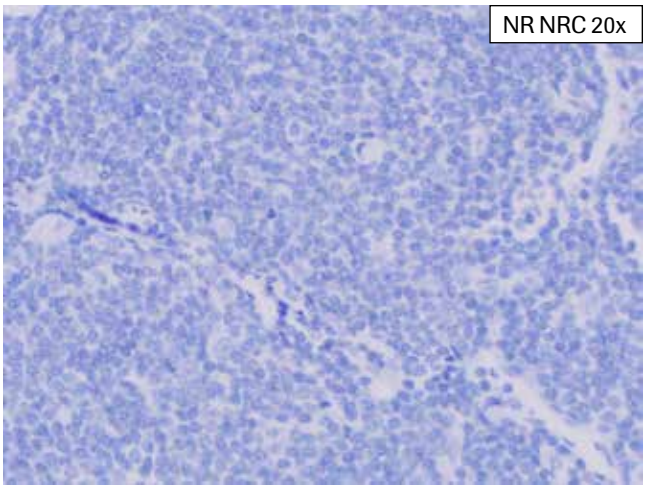


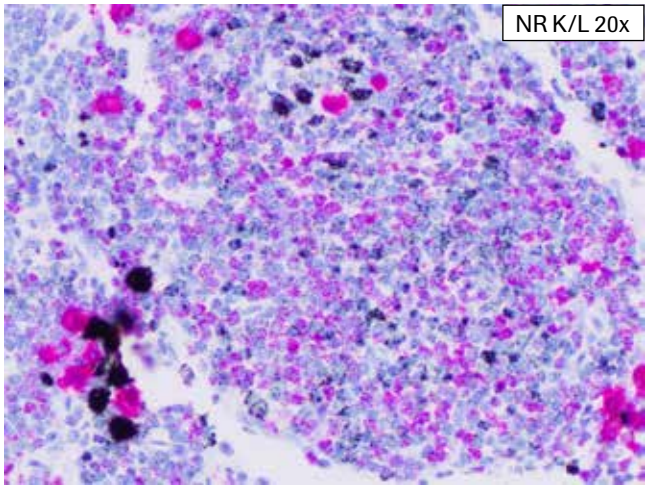
Lambda Restricted – High Expressing: The images above show a plasmacytoma stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe.

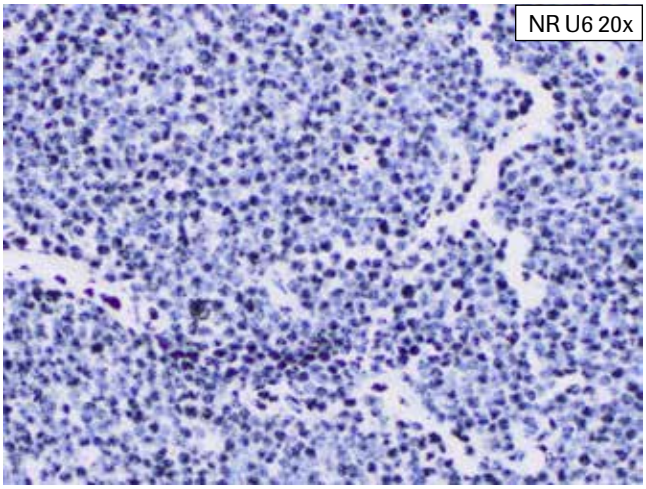
Non-Restricted

Cases with a kappa (magenta): lambda (black) ratio less than 4:1 but are greater than 1:2 are considered non-restricted (NR). These cases show a mixed population of B-cells and plasma cells expressing both kappa and lambda at various expression levels in a normal ratio.









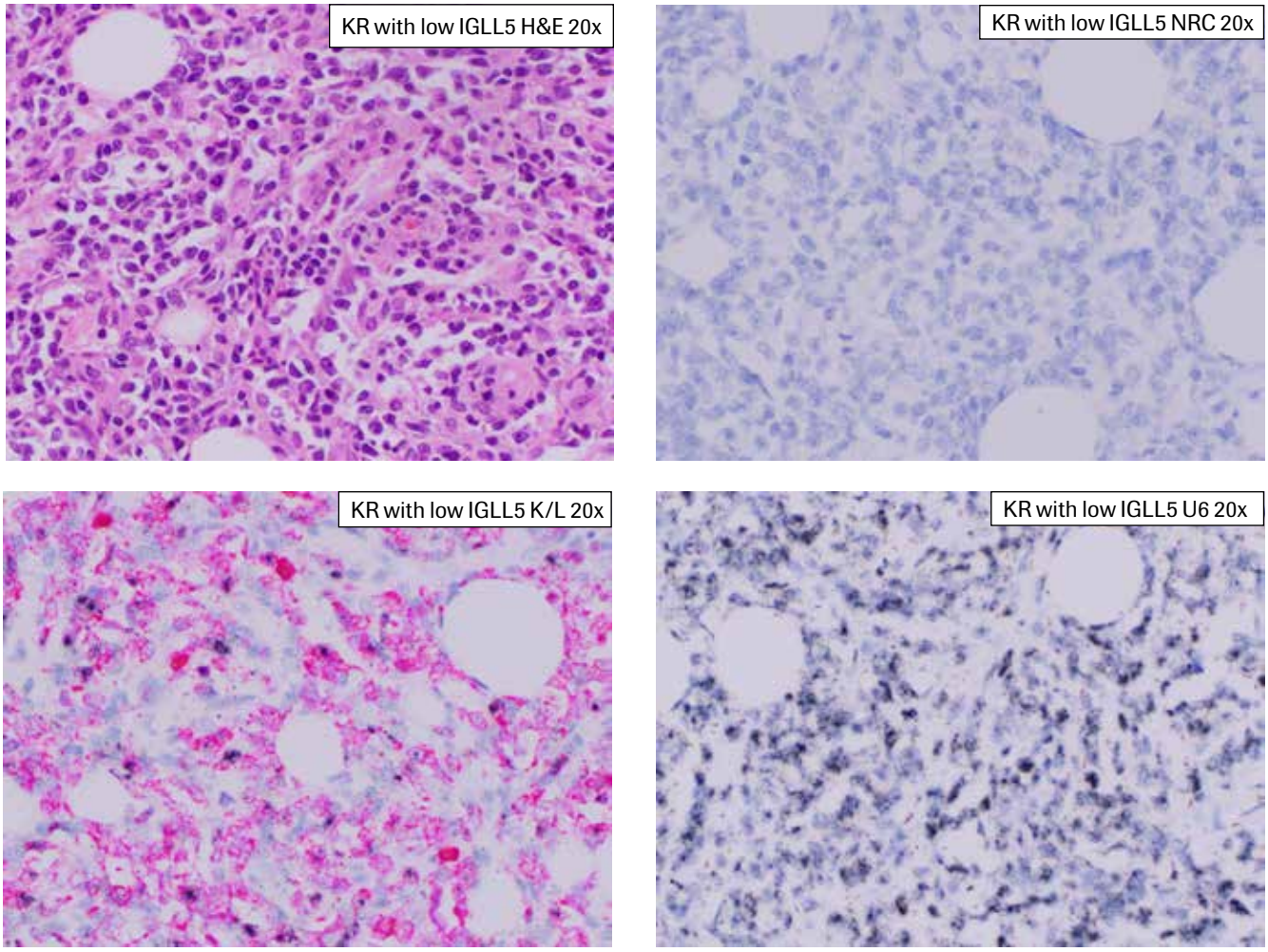
Non-Restricted: The images above show a reactive lymph node stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe. Note the differing expression levels of mRNA in the B-cells and the plasma cells, as well as the mixed population of kappa expressing and lambda expressing cells.

Challenging Cases

IGLL-5 Homology

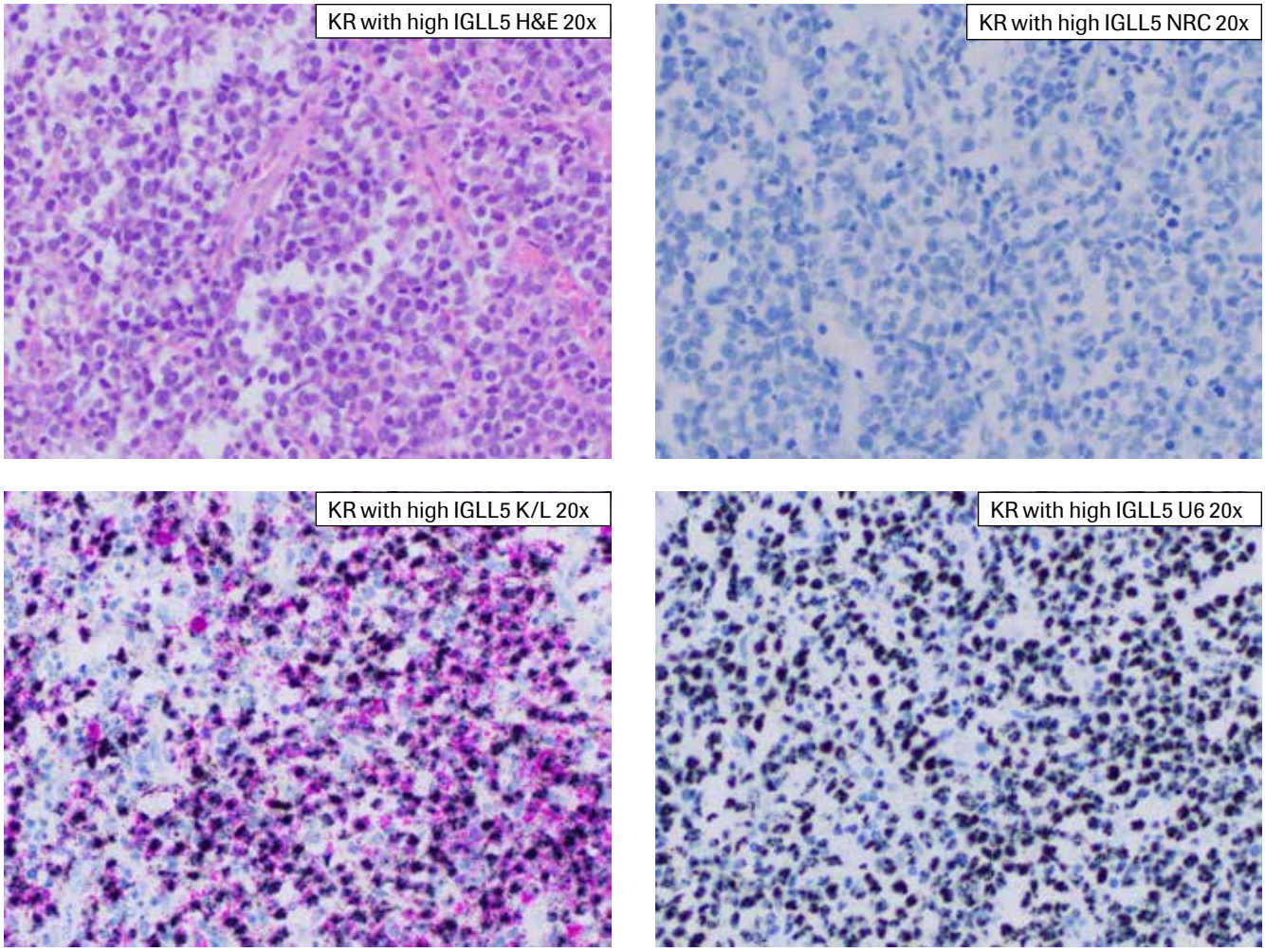
A subset of restricted cases (around 15%) show an abnormal pattern of expression with cytoplasmic and nuclear signal in the same cell[1]. The nuclear signal appears as a silver dot, or cluster of dots, generally larger than the typical mRNA signal. This silver nuclear dot staining is caused by the lambda probe binding to IGLL-5, a nuclear transcript[2]. This transcript has 100% sequence homology to the lambda transcript, therefore this specific off target staining cannot be prevented[1].

Specific off target IGLL-5 staining is most challenging to the interpretation of kappa restricted (KR) cases. In these cases both cytoplasmic kappa and nuclear silver staining will be observed in the same cells. The specific off target nuclear silver staining may be heterogeneous in some cases and only appear in a subset of the restricted cell. Rarely, the specific off-target binding may produce minor cytoplasmic silver staining. In these cases, the specific off target IGLL-5 silver signal should be ignored, and the case should be interpreted as kappa restricted. Care should be taken in interpretation to identify IGLL-5 staining, and distinguish it from non-restricted staining, which shows a mixed population, or dual expressing cases, which are described in a later section.



Low IGLL5: The images above show a DLBCL stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe. This DLBCL is kappa restricted and has low expression of IGLL5.

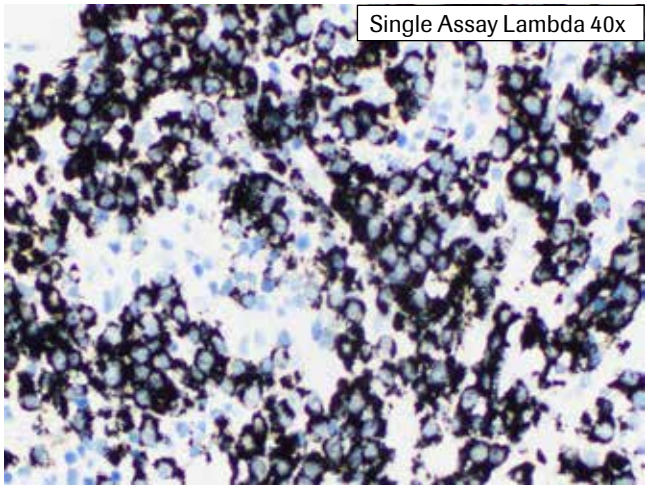
IGLL-5 Homology

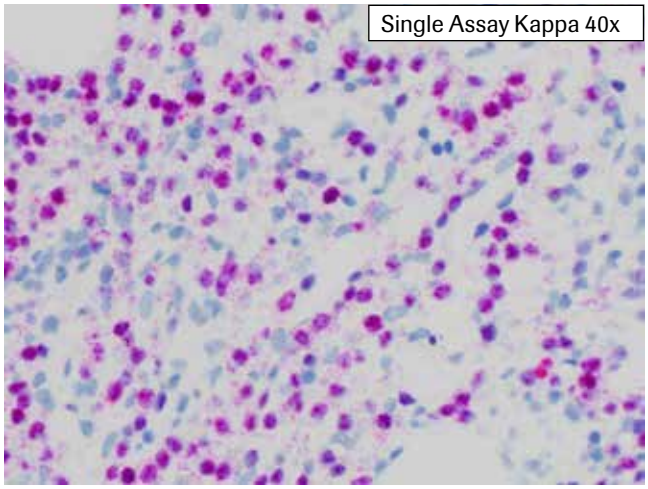


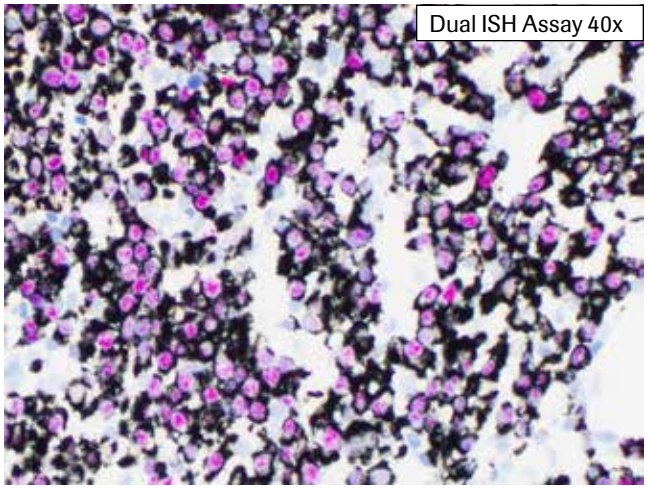
High IGLL5: The images above show a marginal zone lymphoma stained with H&E, Negative Reagent Control, VENTANA K/L Probe Cocktail, and VENTANA U6 BF Probe. This marginal zone lymphoma is kappa restricted and has high expression of IGLL5.

Dual Staining

Although a majority of B-cell lymphomas and plasma cell neoplasms exhibit the conventional staining pattern of kappa OR lambda light chain in each neoplastic cell, a small number of cases (approximately 5%) exhibit a dual staining pattern (kappa and lambda mRNA in the same neoplastic cells). This abnormal dual staining pattern has been verified using kappa only and lambda only assays. You will see from the example below, that the dual ISH assay shows dual staining with a lambda cytoplasmic signal, and a kappa nuclear signal. The kappa only assay shows nuclear kappa staining, while the lambda only assay demonstrate cytoplasmic lambda staining, verifying the presence of both signal in each neoplastic cell.



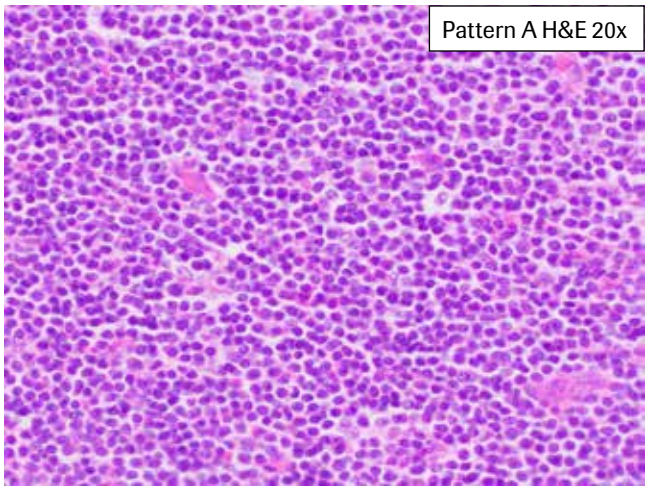


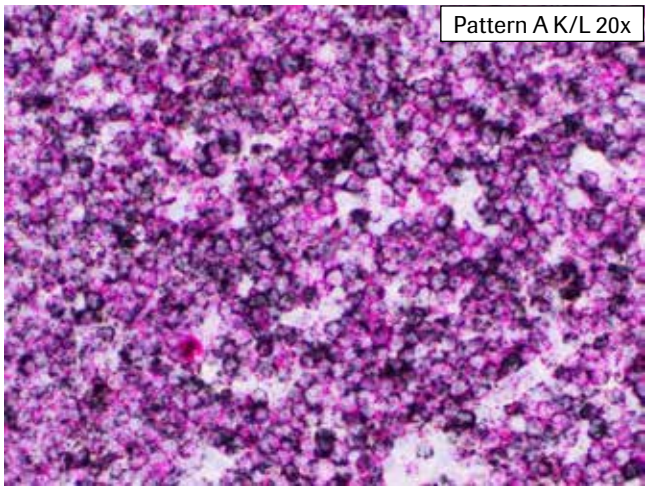


Dual Staining Patterns

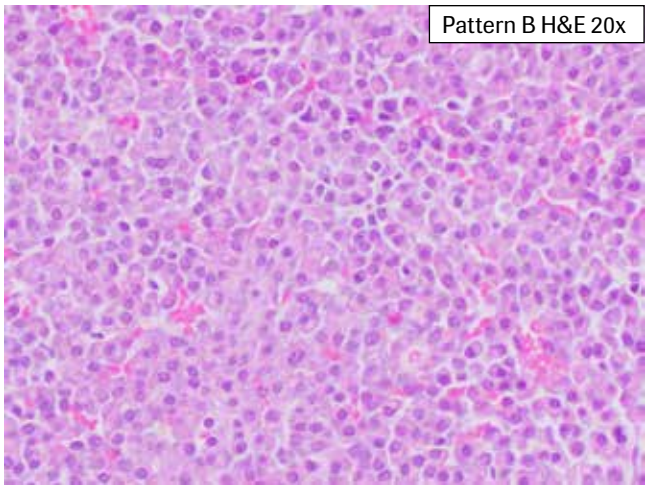
The most dominant dual staining pattern is the presence of cytoplasmic kappa and lambda mRNA, described as “Pattern A” below. There may be differing levels of kappa and lambda expression on the same cells, which may challenge interpretation. The second observed staining pattern is lambda cytoplasmic staining, with nuclear kappa, termed ”Pattern B.” Limited studies have been performed to rule out cross hybridization with kappa DNA, suggesting that the nuclear signal is potentially entrapped kappa RNA.

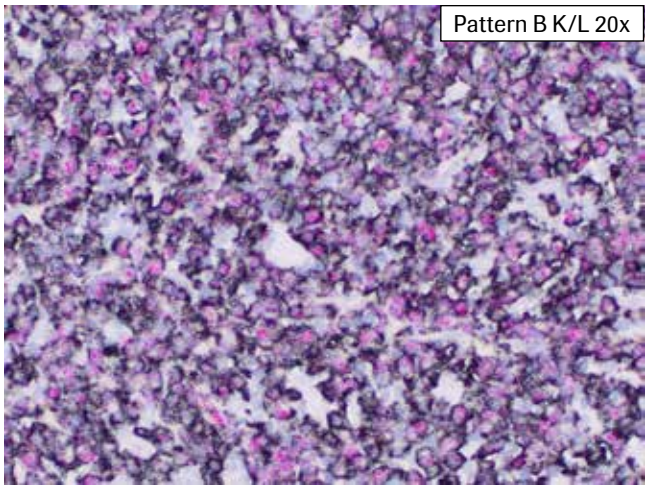
The significance of the dual staining pattern is unknown at this time. This pattern does not appear to be limited to any specific B-cell lymphoma sub-type and also appears in plasma cell neoplasms. The correlation of this staining pattern with light chain restriction status at the protein level is also unclear. At this time, it is recommended to use a protein based assay to determine the light chain restriction in cases showing dual staining pattern.





Dual Staining Pattern A: This is the most common pattern for co-localized staining with the VENTANA K/L Probe Cocktail assay. The image on the right shows the dual staining pattern, while the image on the left shows an H&E of the same case. This pattern is characterized by dual cytoplasmic staining of both kappa and lambda.

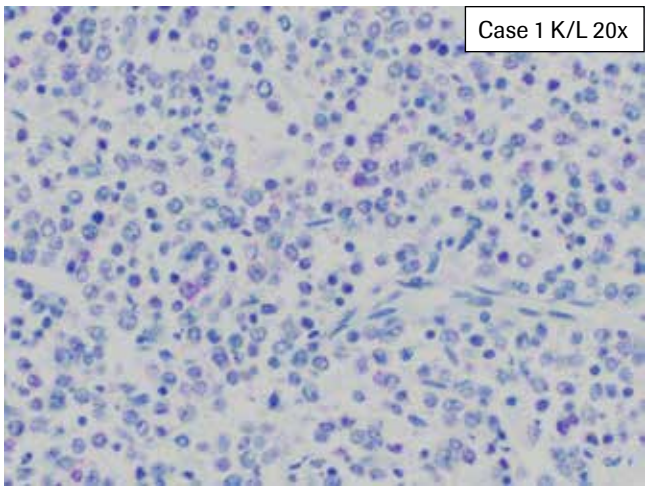


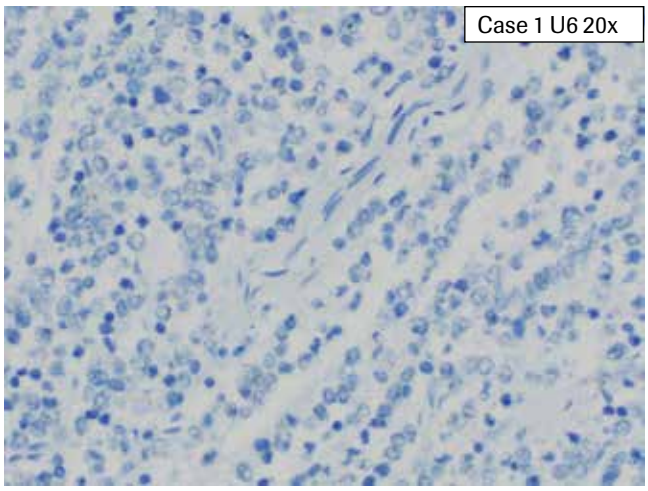


Dual Staining Pattern B: This a less common pattern for co-localized staining with the VENTANA K/L Probe Cocktail assay. The image on the right shows the dual staining pattern, while the image on the left shows an H&E of the same case. This pattern is characterized by lambda cytoplasmic staining with nuclear kappa staining.

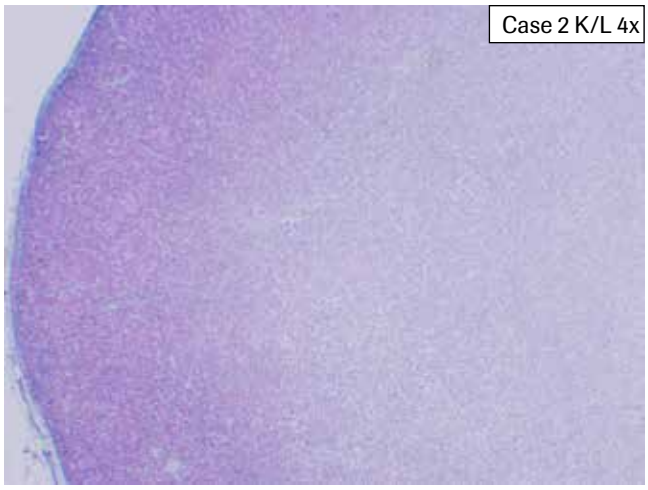
Light Staining

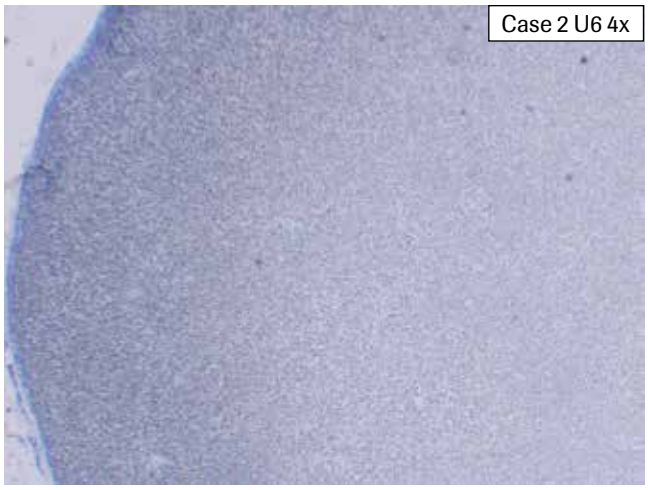
Some cases stained with VENTANA K/L Probe Cocktail assay may have reduced performance on tissues due to RNA degradation. As stated in the "mRNA Integrity Assessment" section, the U6 snRNA transcript can be used as a surrogate to assess RNA integrity. Light staining when using the VENTANA K/L Probe Cocktail assay and the VENTANA U6 BF Probe can indicate that RNA in the target sample is degraded.





Challenging Case: The images on the left show cases stained with VENTANA K/L Probe Cocktail that demonstrate diminished stain intensity in the center portion of the tissue, suggesting a fixation issue. The images on the right are the same cases stained with VENTANA U6 BF Probe to assess mRNA integrity. The lack of appropriate VENTANA U6 BF Probe staining is indicative of possible mRNA degradation.





Challenging Case: This case highlights the use of VENTANA U6 BF Probe to determine areas of poor fixation in a tissue. In the images above you can see the diminished VENTANA K/L Probe Cocktail staining and the diminished VENTANA U6 BF Probe staining, which highlights the fixation defect. Kappa and lambda ratios should only be assessed in areas of preserved mRNA.

References

1. Tubbs RR, Wang H, Wang Z, Minca EC, Portier BP, Gruver AM, Lanigan C, Luo Y, Cook JR, Ma XJ: Ultrasensitive RNA *in situ* hybridization for detection of restricted clonal expression of low-abundance immunoglobulin light chain mRNA in B-cell lymphoproliferative disorders. Am J Clin Pathol. 2013, 140: 736-746.
2. Lisa M Rimsza, William A Day, Sarah McGinn, Anne Pedata, Yasodha Natkunam, Roger Warnke, James R Cook, Teresa Marafioti, and Thomas M Grogan. Kappa and lambda light chain mRNA *in situ* hybridization compared to flow cytometry and immunohistochemistry in B-cell lymphomas. Diagn Pathol. 2014 Jul 21;9:144. doi: 10.1186/1746-1596-9-144.

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

navifyportal.roche.com

102202118EN Rev A

2024-05-01

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