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EBER, Kappa and Lambda CISH probes

The CISH probes and associated detection reagents enable the automated chromogenic visualization of Epstein-Barr Virus (EBV) RNA and Kappa and Lambda mRNA expression on formalin-fixed paraffin-embedded (FFPE) tissue samples.

The probes combine Agilent's oligonucleotide-based technology and formamide-free IQFISH hybridization buffer. IQFISH hybridization buffer is a unique, fast hybridization technology that enables CISH results in about 5 hours. The CISH probes come Ready-to-Use in Dako Omnis vials.

Combining these advancements with the fully automated Dako Omnis provides same-day, hands-free staining.

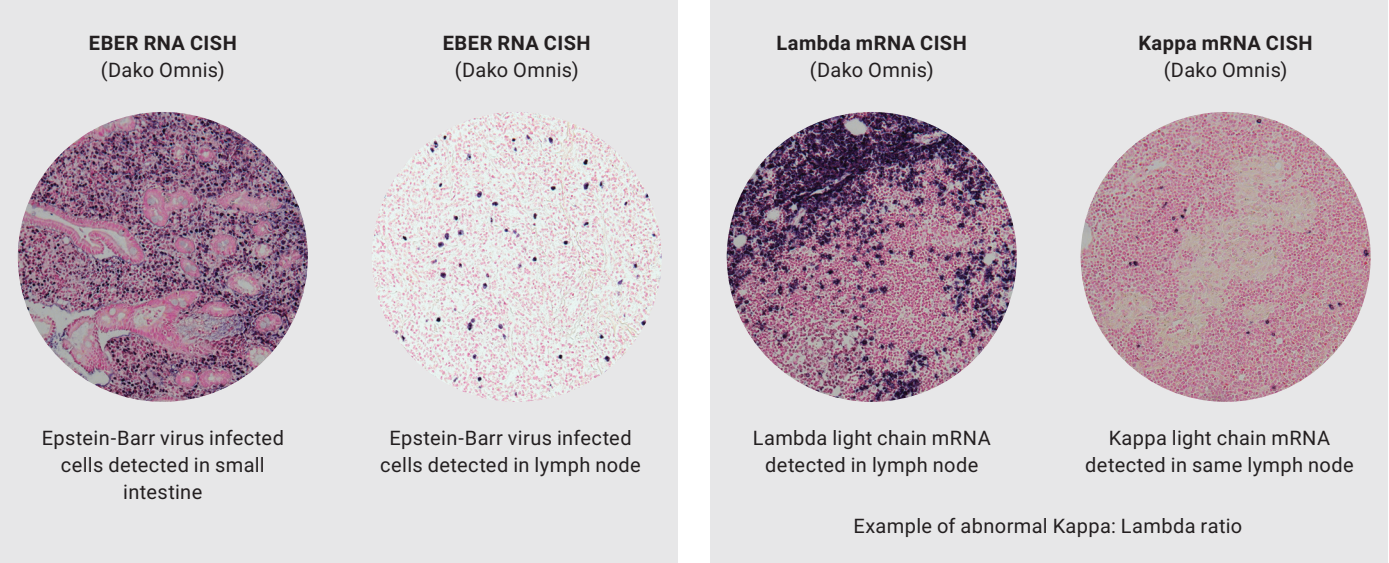
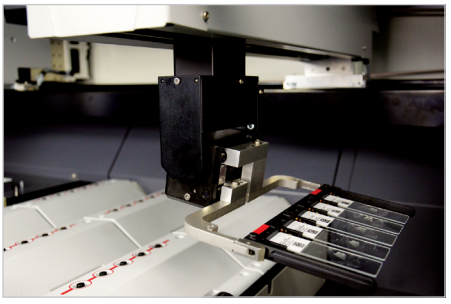


Figure 1. RNA CISH on Dako Omnis.

Automated CISH on Dako Omnis

Dako Omnis, a fully automated, walk-away solution for advanced staining, provides a CISH solution with high efficiency and flexibility. With the short CISH protocol and high slide and reagent capacity, Dako Omnis processes CISH and FISH slides in an IHC-like turnaround time – fast and with high quality.



Principle of the procedure

The oligonucleotide-based CISH probes are conjugated with fluorescein isothiocyanate (FITC) to enable detection using the Anti-FITC-AP CISH Accessory Kit (Dako Omnis). The kit provides all the reagents necessary for chromogenic visualization of Epstein-Barr Virus (EBV) RNA and Kappa and Lambda mRNA expression on formalin-fixed paraffin-embedded (FFPE) tissue samples onboard Dako Omnis.

Anti-FITC/AP CISH Accessory Kit (Dako Omnis):

- CISH Endogenous Enzyme Block (Dako Omnis)
- Anti-FITC-AP (Dako Omnis)
- BCIP-NBT Substrate (Dako Omnis)
- Nuclear Fast Red (Dako Omnis)

Following hybridization with EBER RNA, Kappa or Lambda mRNA probes, CISH Endogenous Enzyme block is added to suppress endogenous enzyme activity followed by incubation with the Anti-FITC-AP primary antibody.

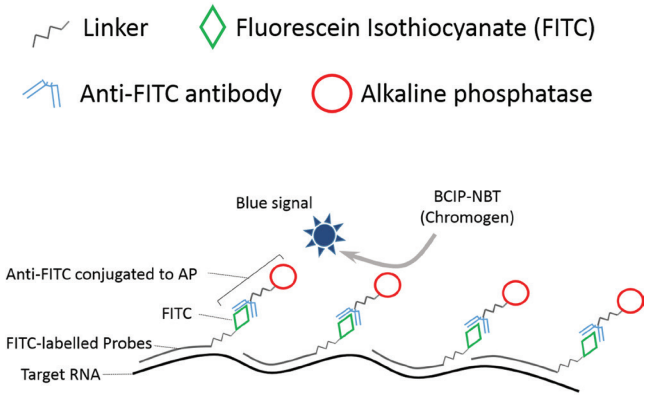


Figure 2. Schematic of method for detection of FITC-labeled probes. Anti-FITC-AP: Alkaline phosphatase-conjugated antibody to fluorescein. BCIP-NBT substrate: 5-bromo-4-chloro-3-indolylphosphate (BCIP) and nitroblue tetrazolium (NBT).

Crisp dark blue/purple staining

The Anti-FITC-AP antibody binds to the FITC molecules on the probe with the conjugated alkaline phosphatase acting to catalyze the deposition of the BCIP-NBT substrate chromogen. The result is crisp dark blue/purple staining at the sites where the probe is bound.

Clear visualization of the tissue morphology

A final incubation with Nuclear Fast Red counterstains the nuclei pink, allowing for the clear visualization of the tissue morphology.

Run CISH simultaneous with FISH and IHC with modest impact on IHC throughput

Flexibility

- Unlike competitor systems, Dako Omnis is designed to enable simultaneous CISH, FISH and IHC runs.
- CISH and FISH slides can be run with modest impact on IHC throughput, as shown in the graph at the bottom.

Figure 3. Number of slides processed in an 8-hour workday.

Add 52 more FISH and CISH days a year

Efficiency

- Start testing and complete review on every weekday – also Friday.
- Five effective FISH and CISH days every week - because time matters.
- Run CISH and FISH whenever it is needed, any day of the week, with little impact on workflow.

Probe summary

EBER RNA CISH (Dako Omnis)

In situ detection of the abundantly expressed EBV-encoded RNA (EBER) genes, can identify lymphoma-associated viral infections, knowledge that can be used to help guide disease management.[1]

EBER RNA CISH (Dako Omnis) is intended for the detection of Epstein-Barr Virus (EBV) RNA. It is an oligonucleotide-based probe that binds to the EBER1 and EBER2 nuclear RNAs. The probe is conjugated with fluorescein isothiocyanate (FITC) to enable detection using the Anti-FITC-AP CISH Accessory Kit (Dako Omnis). EBV-infected cells will exhibit dark blue/purple nuclear staining. It is recommended to be used on formalin-fixed, paraffin-embedded (FFPE) tissue specimens. Positive staining is indicative of latent EBV infection.

Highly specific EBER RNA CISH probe (Dako Omnis)

The probe detects EBER expression in cells of a Hodgkin's Lymphoma lymph node specimen with no background or non-specific signal (figure 5a). No signal is observed in a normal area of the same lymph node (figure 5b).

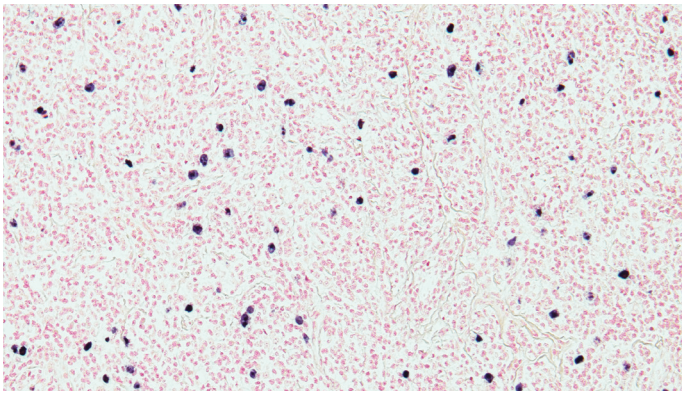


Figure 4a. EBER RNA CISH on Dako Omnis detects Epstein-Barr virus (EBV) infected cells, in lymph node FFPE section from a Hodgkin Lymphoma case. Image at 10X magnification.

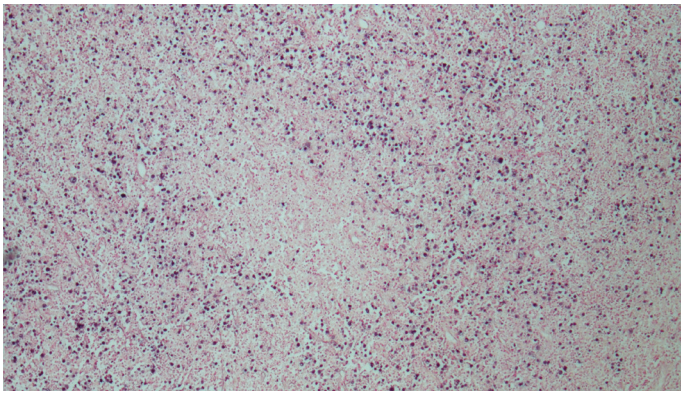


Figure 5a. EBER RNA CISH on Dako Omnis detects 30 – 50% Epstein-Barr Virus (EBV) infected cells in lymph node FFPE section from a Hodgkin Lymphoma case. Image at 4X.

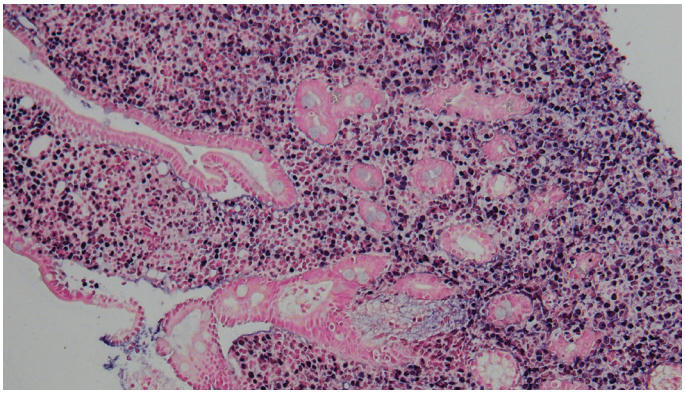


Figure 4b. Large number of EBV infected cells detected in small intestine FFPE section by EBER RNA CISH on Dako Omnis. Image at 10X magnification.

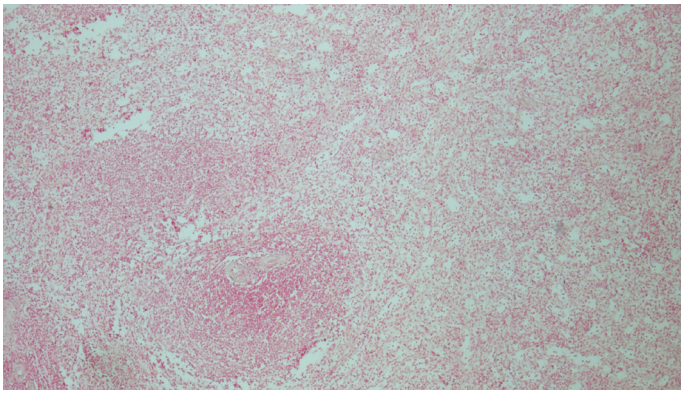


Figure 5b. Same lymph node with area containing a normal lymph node cell population. EBER RNA CISH on Dako Omnis do not generate non-specific signal or background. Image at 4X.

Probe summary

Kappa and Lambda mRNA CISH (Dako Omnis)

In situ mRNA detection of Kappa and Lambda light chain expression is an important tool used in the diagnostic testing of formalin-fixed, paraffin-embedded tissue sections. For example, detection of clonal population of infiltrating plasma cells in bone marrow, expressing either Kappa or Lambda immunoglobulin light chain cytoplasmic mRNA, may serve as a valuable indicator of disease states.

Kappa and Lambda mRNA CISH (Dako Omnis) is intended for the detection of Kappa and Lambda immunoglobulin light chain mRNA. It is an oligonucleotide-based probe conjugated with fluorescein isothiocyanate (FITC) to enable detection using the Anti-FITC-AP CISH Accessory Kit (Dako Omnis). Plasma cells expressing Kappa or Lambda mRNA will exhibit dark blue/purplecytoplasmic staining. It is recommended to be used on formalin-fixed, paraffin-embedded (FFPE) tissue specimens.

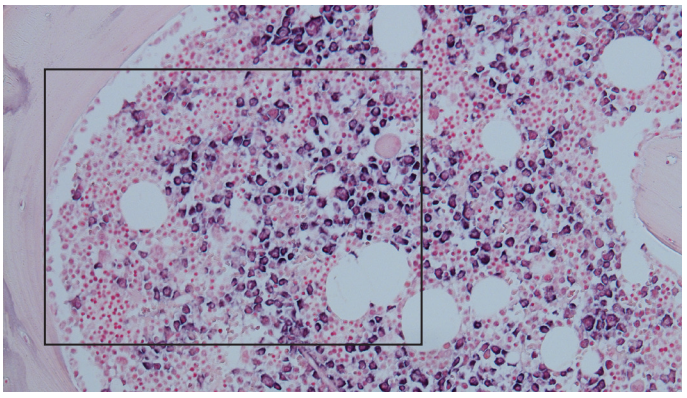


Figure 6a. CISH on Dako Omnis detects Kappa light chain restricted bone marrow trephine (BMT) FFPE section. Image at 10X magnification.

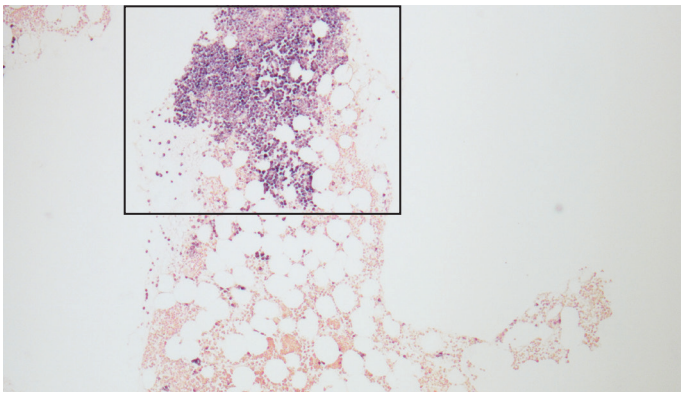


Figure 7a. CISH on Dako Omnis detects Lambda light chain mRNA in this bone marrow trephine (BMT) FFPE section. Image at 4X magnification.

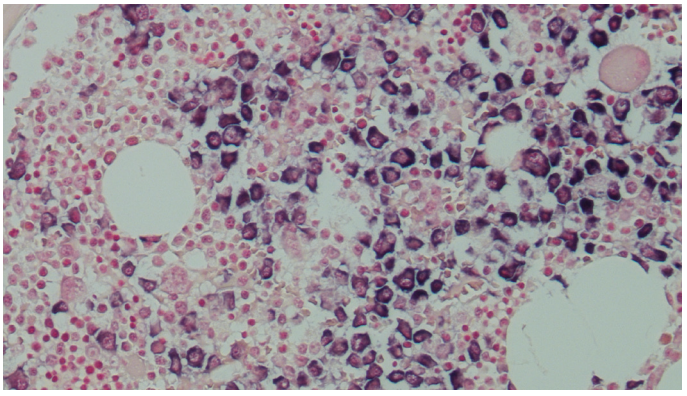


Figure 6b. Boxed area clearly showing cytoplasmic staining at 20X magnification.

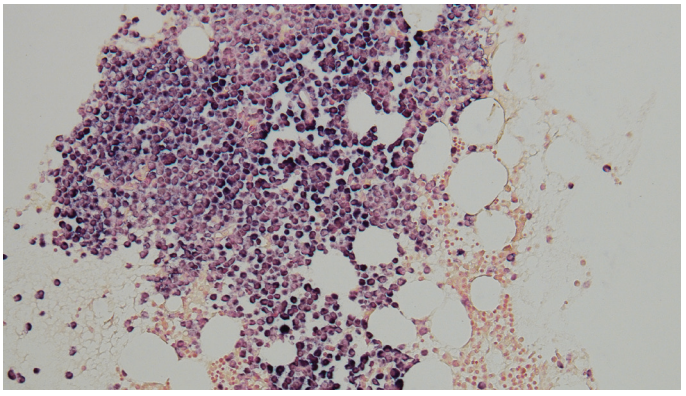


Figure 7b. Box area shown at 10X magnification.

Probes perform well across multiple clinical tissue specimens and varying levels of RNA expression.

EBER RNA CISH (Dako Omnis) detects low, medium and high EBER expression in three different lymph node specimens.

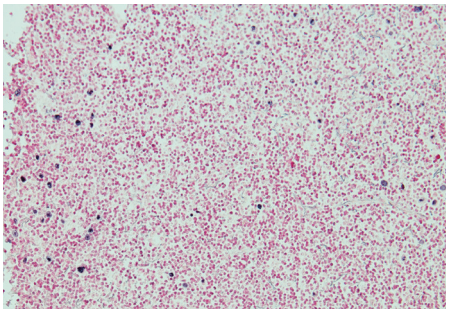


Figure 8a. FFPE lymph node from Hodgkin's Lymphoma case stained with EBER RNA CISH on Dako Omnis. Specimen with LOW number of EBV infected cells.

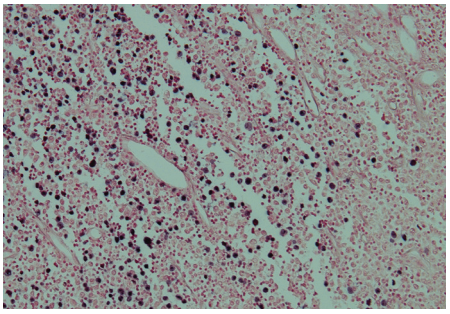


Figure 8b. FFPE lymph node from Hodgkin's Lymphoma case stained with EBER RNA CISH on Dako Omnis. Specimen with MEDIUM number of EBV infected cells.

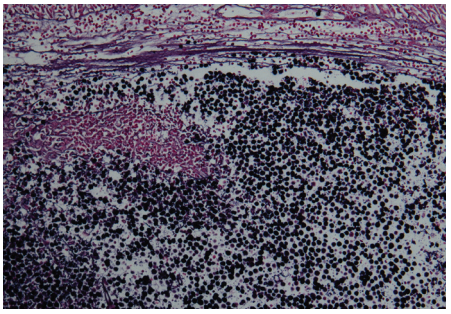


Figure 8c. FFPE lymph node from Hodgkin's Lymphoma case stained with EBER RNA CISH on Dako Omnis. Specimen with HIGH number of EBV infected cells.

Kappa mRNA CISH (Dako Omnis) detects low, medium and high Kappa expression in three different clinical tissue specimens.

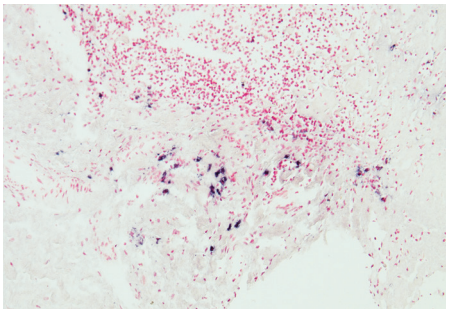


Figure 9a. FFPE lymph node from Non-Hodgkin's Lymphoma case stained with Kappa CISH on Dako Omnis. Specimen with LOW number of positive plasma cells. Image at 10X.

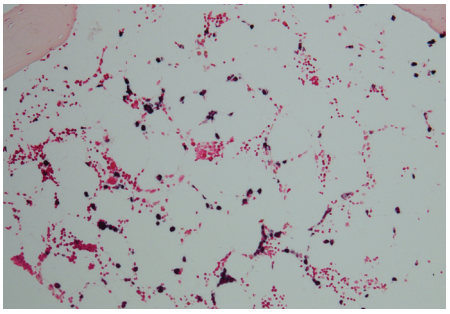


Figure 9b. FFPE BMT from Multiple Myeloma case stained with Kappa CISH on Dako Omnis. Specimen with MEDIUM number of positive plasma cells. Image at 10X.

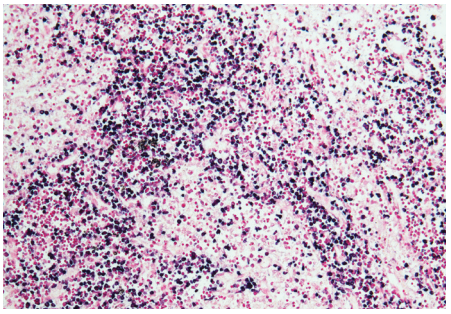


Figure 9c. FFPE lymph node from Non-Hodgkin's Lymphoma stained with Kappa CISH on Dako Omnis. Staining detects HIGH number of Kappa positive plasma cells. Image at 10X.

Lambda mRNA CISH (Dako Omnis) detects low and high Lambda expression.

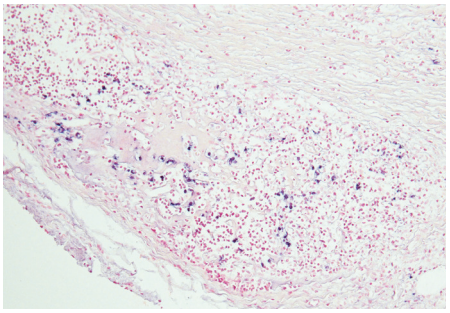


Figure 10a. FFPE lymph node from Non-Hodgkin's Lymphoma case stained with Lambda CISH on Dako Omnis. Specimen with LOW number of positive plasma cells. Image at 10X.

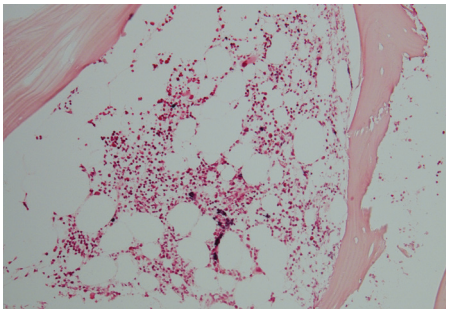


Figure 10b. FFPE BMT specimen stained Multiple Myeloma case with Lambda CISH on Dako Omnis. Specimen with LOW number of positive plasma cells for Lambda light chain mRNA. Image at 10X.

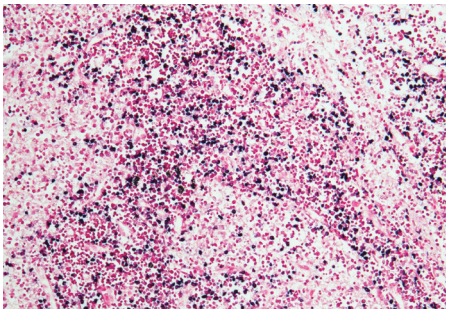


Figure 10c. FFPE lymph node from Non-Hodgkin's Lymphoma stained with Lambda CISH on Dako Omnis. Staining detects HIGH number of Lambda positive plasma cells. Image at 10X.

Positive Control RNA CISH (Dako Omnis)

Positive Control RNA CISH (Dako Omnis) is intended for the evaluation of RNA quality. It is an oligonucleotide-based probe that binds to the 28S ribosomal RNA. The probe is conjugated with fluorescein isothiocyanate (FITC) to enable detection using the Anti-FITC-AP CISH Accessory Kit (Dako Omnis). Cells will have a uniform dark blue/purple staining. It is recommended to be used on formalin-fixed, paraffin-embedded (FFPE) tissue specimens.

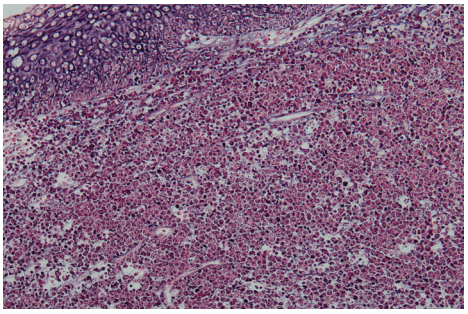


Figure 11a. Positive control (28S rRNA) RNA CISH on Dako Omnis performed on a EBER positive lymph node FFPE section. Image at 10X magnification.

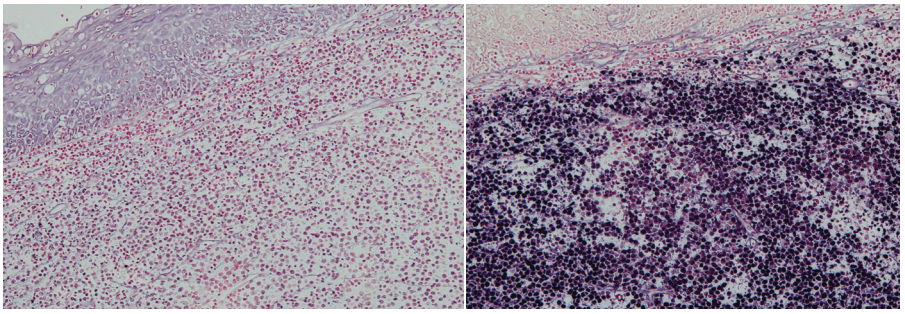


Figure 11b. To simulate conditions wherein sample RNA integrity is compromised, lymph node FFPE section was treated with RNase A/T1 enzyme mix to degrade RNA, prior to performing Positive control RNA CISH on Dako Omnis. Compromised 28S rRNA signal correlates with compromised RNA integrity due to RNase treatment. Image at 10X magnification (left image). Epstein-Barr virus (EBV) infected cells, in the same lymph node FFPE section, detected by EBER RNA CISH on Dako Omnis. Image at 10X magnification (right image).

Robust CISH protocol

The CISH probes have been validated using the default Agilent protocol shown in Table 1 and are proven robust across multiple tissue specimens and varying levels of RNA expression.

Step	Reagent	Time and Temperature
Dewaxing	Clearify	10 minutes
Target retrieval	ISH Pre-Treatment Solution (Dako Omnis)	3 minutes*, 70°C*
Wash	ISH Ethanol Solution, 96% (Dako Omnis)	2 x 3 minutes
Digestion	ISH Pepsin (Dako Omnis)	15 minutes*
Drying	—	15 minutes, 45°C
Denaturation	—	10 minutes, 66°C
Hybridization	CISH (Dako Omnis) probe	60 minutes, 45°C
Stringent wash	ISH Stringent Wash Buffer (Dako Omnis)	3 minutes*, 55°C*
Endogenous enzyme blocking	CISH Endogenous Enzyme Block (Dako Omnis)	3 minutes*
Antibody binding	Anti-FITC-AP (Dako Omnis)	30 minutes*
Chromogen addition	BCIP-NBT Substrate (Dako Omnis)	2 x 20 minutes*
Counterstaining	Nuclear Fast Red (Dako Omnis)	10 minutes*

Table 1. Simplified overview of the automated staining protocol for Agilent CISH probes performed onboard Dako Omnis. *Step is modifiable. Any changes made to the Agilent protocol above must be validated by the user.

Ordering information

	Product Name	Code	Vol. & Test per Unit
CE	Kappa and Lambda mRNA CISH (Dako Omnis)*	G111700-2	2 mL, RTU, 20 tests
CE	EBER RNA CISH (Dako Omnis)*	G111701-2	2 mL, RTU, 20 tests
CE	Positive Control RNA CISH (Dako Omnis)*	G111702-2	2 mL, RTU, 20 tests
CE	Anti-FITC/AP CISH Accessory Kit (Dako Omnis)*	K589911-2	40 tests, 4 vials, RTU
CE	ISH Ethanol Solution, 96% (Dako Omnis) **	GM30011-2	14 mL, RTU, 20 tests
CE	ISH Pre-Treatment Solution (20x) (Dako Omnis) **	GM30111-2	175 mL for 3.5 L bulk, 5-25 tests
CE	ISH Pepsin (Dako Omnis)**	GM30211-2	7 mL, RTU, 20 tests
CE	FISH Stringent Wash Buffer (20x) (Dako Omnis)**	GM30311-2	175 mL for 3.5 L bulk, 5-25 tests
CE	ISH Cleaning Solution (Dako Omnis)**	GC20730-2	10 mL, RTU, 100 tests
	Dako Omnis FISH Lid**	GC10230	Box of 5, 25 tests
CE	Dako Omnis Mixing Device**	GC20630	1 pcs

* Manufactured in Cedar Creek, Texas
** Manufactured in Glostrup, Denmark

Companion devices

Dako Omnis Mixing Device

Dako Omnis FISH Lids

References

(1) Carbone A, Gloghini A, Dotti G. EBV-associated lymphoproliferative disorders: classification and treatment. Oncologist. 2008 May;13(5):577-8.

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