DATA SHEET-V1
REMBRANDT® EBER RISH
DETECTION

CE IVD

Pan Path

Ref		
A500K.0101	Σ	10-100 T
A500K.0105	Σ	10-100 T
A500K.9901	Σ	10-100 T
A500K.9905	Σ	10-100 T

Intended use

i) The REMBRANDT® EBER RISH detection assay is an in-vitro diagnostics medical device intended for the detection of EBER1 and EBER2 miRNA by means of chromogenic RNA *in situ* hybridization.

ii) The REMBRANDT® EBER RISH detection assay is intended for the EBER1 and EBER2 miRNA in fixed cells and FFPE tissue sections. The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration clinical history, symptoms, as well as other possible test data.

iii) The REMBRANDT® EBER RISH detection assay kit is a qualitative assay for the detection of EBER1 and EBER2 miRNA.

iv) The intended users are qualified laboratory employees in cytology and/or pathology. The product is intended for professional use.

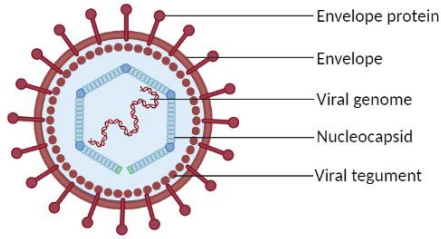
Clinical relevance

The causative agent of Pfeiffer's disease is the Epstein-Barr virus (EBV). The EBV genome consists of linear double-stranded DNA that encodes at least thirty polypeptides. Epstein-Barr virus (EBV) is found throughout all human populations with a prevalence of 90% in adults. Primary EBV infections usually occurs asymptomatic in childhood and latently persists during life-time. The EBV virus is directly associated with human cancers such as Burkitt's lymphoma, Post-transplant lymphoproliferative disease-like lymphomas in immune-comprised individuals (transplant patients and persons infected with AIDS), Hodgkin disease and nasopharyngeal carcinoma which is a common carcinoma in South East Asia (Kuri et al., 2020). At least 11 EBV genes are expressed during latent infection. Two of those are small non-coding RNAs (EBER 1 and EBER 2), six encode nuclear proteins (EBNAs and LP) and three encode membrane proteins (LMPs) (Yoshizaki et al., 2013). The EBER genes are transcribed by RNA polymerase and are the most abundant EBV transcripts in Burkitt lymphoma and other latent infected cells. In Burkitt lymphoma, the EBV virus up-regulates BCL-2 in concert with a down regulation of c-Myc causing inhibition of apoptosis, thus promoting tumour genesis.

EBV infects humans via the lymphoid tissue of the oropharynx. Here first the epithelial cells and then the B cells are infected (Sheikh & Qadri, 2011).

Probe specification

The REMBRANDT® EBER RISH detection assay is designed to target the EBER1 and EBER2 miRNA in an active EBV infection by means of chromogenic RNA in situ hybridization (RISH). The complementary RNA probes are 5' and 3' labelled with digoxigenin or biotin.
The REMBRANDT® EBER RISH probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.



Test principle

In a chromogenic RNA in situ hybridization assay, a single stranded RNA oligonucleotide probe labelled with a hapten (digoxigenin or biotin) is used. The labelled oligonucleotide probe is diluted in a hybridization mixture. The hybridization mixture containing the oligonucleotide probe is added to the specimen. The oligonucleotide probe RNA is able to hybridize to its complementary target sequences in the cells. The haptens need to be detected using conjugated antibodies. The HRP or AP conjugated antibodies are able to attach to the haptens located at the 5' and '3 of the oligonucleotide probe. After incubation with the corresponding antibodies, a chromogenic-substrate reaction will allow visualization of EBER miRNA via bright field microscopy.

Reagents provided

Product name	Product number	Amount
Labelled probe (depending on label)		
● REMBRANDT® EBER RISH probe BIO	A500P.0100	Σ 1 mL
detection REMBRANDT® RISH negative control BIO probe	Q101P.0100	Σ 1 mL

REMBRANDT® RISH positive control BIO probe	Q152P.0100	Σ 1 mL
● REMBRANDT® EBER RISH probe DIG detection	A500P.9900	Σ 1 mL
REMBRANDT® RISH negative control DIG probe	Q101P.9900	Σ 1 mL
REMBRANDT® RISH positive control DIG probe	Q152P.9900	Σ 1 mL

Conjugated antibodies (depending on probe and detection system)

● REMBRANDT® aDIG-AP conjugate	R003R.0000	15 mL
● REMBRANDT® aDIG-HRP conjugate	R004R.0000	15 mL
● REMBRANDT® aBIO-AP conjugate	R041R.0000	15 mL
● REMBRANDT® aDIG-HRP conjugate	R042R.0000	15 mL

Chromogen substrate and counterstaining (depending on detection system)

●AEC substrate and	R007R.0000	2 mL
AEC buffer	R010R.0000	15 mL
REMBRANDT® Methyl green counterstain	R016R.0000	15 mL
●NBT/BCIP	R008R.0000	15 mL
REMBRANDT® Nuclear fast red counterstain	R015R.0000	15 mL

REMBRANDT® Pepsin powder	R011R.0000	1 g
REMBRANDT® Pepsin diluent	R018R.0000	15 ml
REMBRANDT® Positive control slides, paraffin-embedded	Q300C.0000	2 pcs

Assay procedure

REMBRANDT® EBER RISH detection assay procedure for cytological specimen, frozen sections and FFPE tissue sections.

I. *Cytological specimen:*
Deposit the cells on coated glass slides and air dry for 30 minutes. Fix the slides with a cross-linking fixative (e.g. 4% para-formaldehyde) for 10 minutes at room temperature and rinse with PBS.
FFPE tissue sections:
For a detailed description of sample preparation and pre-treatment see: 2.1 Specimen collection and 2.2 Specimen pre-treatment of the PanPath RISH Manual.

II. Incubate both test and control slides (Q300C.0000) in pre-heated proteolytic work solution (prepare according to section 1.9 of RISH manual) (R011R.000 + R018R.000) at 37 ˚C. Paraffin-embedded sections (2.50 mg/ml), cytological specimen (100 µg/ml) or frozen sections (50 µg/ml) for 15 minutes.

III. Flush wash slides in deionised water, followed by dehydration in graded ethanol series (ethanol 70%, 96%, 96%, 100%, 100%) 1 minute each and air-dry slides for 15 minutes.

Do not treat more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete pre-treatment. Additionally, allow the slides to air-dry as recommended; otherwise sections will be lost.

IV. Homogenize probe solutions (A500P.XXXX) and spin briefly. Apply 1 drop of probe solution to each specimen (including control specimen (Q300C.0000)). Cover all specimens with a cover slip (avoid air bubbles).

V. Transfer the slides into a moist environment and incubate for 16 hours at 37 ˚C.

VI. Remove coverslips by soaking the slides in TBS buffer solution at room temperature.

VII. Take the slides out, wipe off excess buffer and dry the edges using a lint-free cloth.

VIII. Apply 2-3 drops of the appropriate conjugate (R0XXR.0000) to each specimen and transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate for 30 minutes.

In case of HRP detection, prepare AEC reaction mixture according to section 2.4 of the RISH manual.

IX. Tap off excess detection reagent and rinse the slides in TBS buffer at room temperature.

X. Transfer the slides into a container with deionised water and soak for 1 minute.

XI. Tap off excess water and dry around the edges using a lint-free cloth. Ensure that the specimen on the slide is not disrupted.

XII. Apply 2-3 drops of the approriate chromogen substrate reaction mixture (according to detection system, see RISH manual section 2.4) to each specimen. Transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate in the dark for 5-15 minutes.

XIII. Tap off excess substrate solution and rinse slides 3 times for 1 minute in fresh deionised water. The slides are now ready to be mounted or counterstained.

When a contrast colour is desired, the slides can be counterstained. If not, proceed to step XVI

XIV. Wipe off excess reagent and apply 2-3 drops of approriate counterstain to each specimen (see RISH manual section 2.5). Incubate for

at least 1 minute (longer incubation is possible and will yield in stronger staining).

XV. Tap off excess counterstain and rinse the slides briefly in deionised water.

XVI. Mount the slides by using an aqueous mounting medium. Interpret the results under a brightfield microscope.

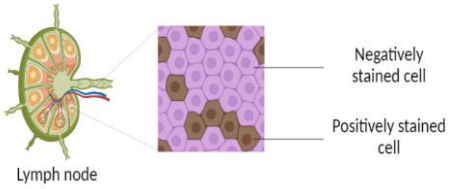
Interpretation of results

Hybridization of the REMBRANDT® EBER RISH probe is viewed using a brightfield microscope. The detection of the EBER1 and EBER2 miRNA is conducted by microscopic examination of cells. The stained cells that contain EBER1 and EBER2 miRNA sequences, stand out brightly against the de-stained cells. The REMBRANDT® EBER RISH detection assay procedure enables visual detection of EBV infected cells. Each signal corresponds with EBER miRNA. First, check if the positive control shows colour precipitations in conformity with the localisation of the target RNA. In the test slides, start under low power magnification and focus on localisation and colour to see whether:

•The positivity (colour precipitation) is observed in conformity with the localisation of the target RNA.
•The colour has the right shade (no endogenous or formalin pigment)

Use high power magnification to see whether:

•The positive staining texture (granular, etc), demarcation and localisation are conform the positive control staining pattern.



For additional signal interpretation see RISH manual section 3.1.

Performance characteristics

Analytical Sensitivity and Specificity

The analytical sensitivity, specificity and precision were investigated within PanPaths analytical performance assessment. The results of the analytical performance study of the REMBRANDT® EBER RISH assay are available upon request.

Clinical performance

The clinical sensitivity was determined for the REMBRANDT® EBER RISH detection assay based on assessment of EBV positive ISH in samples (n=50) with a known EBV status. Clinical specificity was demonstrated by using samples (n=16) infected by similar viruses: HSV1, HSV2, VZV and CMV. The REMBRANDT® EBER RISH detection assay showed to have a clinical sensitivity and specificity of 100% on FFPE tissue sections.

Limitations of Procedure

i) The REMBRANDT® EBER RISH detection assay is solely applicable for the detection of EBER1 and EBER2 which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples or FFPE tissue sections).

ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. In tissue sections are required, the sections should be prepared in a 4 µm thickness. Furthermore, the tissues should be glued to the glass slides with a bio-adhesive (e.g. organ silane), dried at room temperature, subsequently dried at 37 °C overnight and lastly completely deparaffinized in xylene and alcohol series and air dried.

iii) Cytological specimen should be prepared as required by the user, fixed with cytological fixation agent, rinsed in distilled water prior to the ISH procedure and air dried.

iv) Many factors can influence the performance of the ISH procedure. Failure in detection can be due to i.e. improper sampling, handling, the time lapse between tissue removal and fixation, the size of the tissue specimen in the fixation medium, the fixation time, processing fixed tissue, the thickness of the section, the bio-adhesive on the slide, deparaffinisation procedure, incubation times, proteolytic pre-treatment, detection reagents, incubation temperatures and interpretation of results.

v) The performance of the RISH procedure is also affected by the sensitivity of the method and the presence of EBER1 and EBER2 miRNA. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® EBER RISH detection assay results should not be relied on in case the sampling, sampling method, sample quality, sample preparation, reagents used, controls and procedure followed are not optimal or as described the working protocols.

vii) The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history as well as data obtained from other molecular test (i.e. PCR).

viii) Therapeutic considerations based on the result of this test alone should not been taken. Results should be verified by other traditional diagnostic methods such as

but not limited to clinical history, symptoms, as well as morphological data.
ix) The medical profession should be aware of risks and factors influencing the chromogenic signal intensity while interpretating the test result. Microscopy settings might influence the signal intensity and/or interpretation.
x) Laboratory personnel performing the test should be trained and knowledgeable to be able to interpret the test results.

Storage and handling

Store kit and its contents at 2-8°C. Store the dissolved and aliquoted reagents at recommended temperatures. When used and stored as indicated, the kit is stable until the expiry date printed on the box.

Product	Product number	Storage conditions
REMBRANDT® …. RISH probe BIO or DIG detection	XXXP.XX XX	2-8 ˚C
REMBRANDT® Pepsin powder	R011R.00 00	Powder: 2-25˚C, ambient temperature Dissolved: -20˚C
REMBRANDT® Pepsin diluent	R018R.00 00	Concentrated solution and diluted: 2-25˚C, ambient temperature
REMBRANDT® Conjugated antibodies	R0XXR.0 000	2-8 ˚C
REMBRANDT® TBS buffer pouch	R017R.00 00	Powder: 2-25 ˚C Dissolved: 2-25 ˚C
REMBRANDT® NBT/BCIP substrate	R008R.00 00	2-8 ˚C
REMBRANDT® AEC substrate	R007R.00 00	2-8 ˚C
REMBRANDT® AEC buffer	R010R.00 00	2-8 ˚C
REMBRANDT® Nuclear fast red counterstain	R015R.00 00	2-8 ˚C
REMBRANDT® Methyl green counterstain	R016R.00 00	2-8 ˚C
Positive control slides, paraffin embedded	Q300C.00 00	2-25 ˚C

Hazard statements

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H351 - Suspected of causing cancer
H360D - May damage the unborn child
H373 - May cause damage to organs through prolonged or repeated exposure

Precautionary Statements

P202 - Do not handle until all safety precautions have been read and understood
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352 - IF ON SKIN: Wash with plenty of water and soap
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P362 + P364 - Take off contaminated clothing and wash it before reuse
P405 - Store locked up

Additional information

Product in combination with other devices

The REMBRANDT® RNA probes are intended for stand-alone usage. The *in vitro* diagnostic is intended to be used in combination with standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), proteolytic-, detection- and other reagents (not supplied with this reagent) and a microscope. The combination has been tested and validated. Since the standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature controls, incubation time control(s) and other not supplied reagents such as but not limited to proteolytic reagents, detection reagents and a microscope is not combined with the device as a product, conformity with the essential requirements is not applicable. Assay validation criteria are mentioned in *'Interpretation of the Results'* and are also depending on clinical state of the sample, which may influence the validation criteria.

For additional information regarding the REMBRANDT® assays, a manual is included which specifies the following subjects:

- Controls
- Materials required but not included
- Storage and shelf-life
- Performance precautions
- Preparations of reagents
- Specimen collection
- Quality control
- Trouble shooting guide

Technical assistance

For technical assistance regarding the products performance, please contact info@panpath.nl or call +31 495499090. Visit our website for reprints of datasheets or additional documentation. www.panpath.nl

Literature list

Abraham, R. T., & Weiss, A. (2004). Jurkat T cells and development of the T-cell receptor signalling paradigm. *Nature Reviews Immunology*, *4*(4), 301–308. https://doi.org/10.1038/nri1330

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Sheikh, T. I., & Qadri, I. (2011). Expression of EBV Encoded viral RNA 1, 2 and anti-inflammatory Cytokine (interleukin-10) in FFPE lymphoma specimens: A preliminary study for diagnostic implication in Pakistan. *Diagnostic Pathology*, *6*(1), 70. https://doi.org/10.1186/1746-1596-6-70

Yang, H. J., Huang, T. J., Yang, C. F., Peng, L. X., Liu, R. Y., Yang, G. Da, Chu, Q. Q., Huang, J. L., Liu, N., Huang, H. B., Zhu, Z. Y., Qian, C. N., & Huang, B. J. (2013). Comprehensive profiling of Epstein-Barr virus-encoded miRNA species associated with specific latency types in tumor cells. *Virology Journal*, *10*(1), 1. https://doi.org/10.1186/1743-422X-10-314

Yee, C., Krishnan-Hewlett, I., Baker, C. C., Schlegel, R., & Howley, P. M. (1985). Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. *American Journal of Pathology*, *119*(3), 361–366.

Yoshizaki, T., Kondo, S., Wakisaka, N., Murono, S., Endo, K., Sugimoto, H., Nakanishi, S., Tsuji, A., & Ito, M. (2013). Pathogenic role of Epstein-Barr virus latent membrane protein-1 in the development of nasopharyngeal carcinoma. *Cancer Letters*, *337*(1), 1–7. https://doi.org/10.1016/j.canlet.2013.05.018

Disclaimer: This document is valid until the product expiry on the kit label

DATA SHEET-V1
REMBRANDT® EBER RISH
DETECTION

RUO

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A500K.9901	Σ	10-100 T
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Intended use

i) The REMBRANDT® EBER RISH detection assay is intended for the detection of EBER1 and EBER2 miRNA by means of chromogenic RNA *in situ* hybridization.

ii) The REMBRANDT® EBER RISH detection assay is intended for the EBER1 and EBER2 miRNA in fixed cells and FFPE tissue sections. The clinical interpretation of the results should not be established on the basis of this test result.

iii) detection assay kit is a qualitative assay for the detection of EBER1 and EBER2 miRNA.

iv) The intended users are qualified laboratory employees in cytology and/or pathology. The product is intended for professional use.

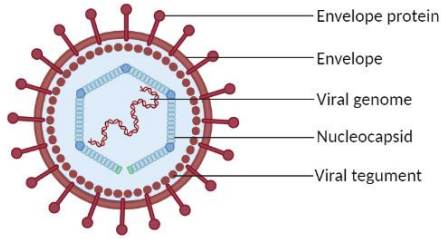
Clinical relevance

The causative agent of Pfeiffer's disease is the Epstein-Barr virus (EBV). The EBV genome consists of linear double-stranded DNA that encodes at least thirty polypeptides. Epstein-Barr virus (EBV) is found throughout all human populations with a prevalence of 90% in adults. Primary EBV infections usually occurs asymptomatic in childhood and latently persists during life-time. The EBV virus is directly associated with human cancers such as Burkitt's lymphoma, Post-transplant lymphoproliferative disease-like lymphomas in immune-comprised individuals (transplant patients and persons infected with AIDS), Hodgkin disease and nasopharyngeal carcinoma which is a common carcinoma in South East Asia (Kuri et al., 2020). At least 11 EBV genes are expressed during latent infection. Two of those are small non-coding RNAs (EBER 1 and EBER 2), six encode nuclear proteins (EBNAs and LP) and three encode membrane proteins (LMPs) (Yoshizaki et al., 2013). The EBER genes are transcribed by RNA polymerase and are the most abundant EBV transcripts in Burkitt lymphoma and other latent infected cells. In Burkitt lymphoma, the EBV virus up-regulates BCL-2 in concert with a down regulation of c-Myc causing inhibition of apoptosis, thus promoting tumour genesis.

EBV infects humans via the lymphoid tissue of the oropharynx. Here first the epithelial cells and then the B cells are infected (Sheikh & Qadri, 2011).

Probe specification

The REMBRANDT® EBER RISH detection assay is designed to target the EBER1 and EBER2 miRNA in an active EBV infection by means of chromogenic RNA in situ hybridization (RISH). The complementary RNA probes are 5' and 3' labelled with digoxigenin or biotin.
The REMBRANDT® EBER RISH probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.



Test principle

In a chromogenic RNA in situ hybridization assay, a single stranded RNA oligonucleotide probe labelled with a hapten (digoxigenin or biotin) is used. The labelled oligonucleotide probe is diluted in a hybridization mixture. The hybridization mixture containing the oligonucleotide probe is added to the specimen. The oligonucleotide probe RNA is able to hybridize to its complementary target sequences in the cells. The haptens need to be detected using conjugated antibodies. The HRP or AP conjugated antibodies are able to attach to the haptens located at the 5' and '3 of the oligonucleotide probe. After incubation with the corresponding antibodies, a chromogenic-substrate reaction will allow visualization of EBER miRNA via bright field microscopy.

Reagents provided

Product name	Product number	Amount
Labelled probe (depending on label)		
● REMBRANDT® EBER RISH probe BIO detection	A500P.0100	Σ 1 mL
REMBRANDT® RISH negative control BIO probe	Q101P.0100	Σ 1 mL

REMBRANDT® RISH positive control BIO probe	Q152P.0100	Σ 1 mL
● REMBRANDT® EBER RISH probe DIG detection	A500P.9900	Σ 1 mL
REMBRANDT® RISH negative control DIG probe	Q101P.9900	Σ 1 mL
REMBRANDT® RISH positive control DIG probe	Q152P.9900	Σ 1 mL

Conjugated antibodies (depending on probe and detection system)

● REMBRANDT® aDIG-AP conjugate	R003R.0000	15 mL
● REMBRANDT® aDIG-HRP conjugate	R004R.0000	15 mL
● REMBRANDT® aBIO-AP conjugate	R041R.0000	15 mL
● REMBRANDT® aDIG-HRP conjugate	R042R.0000	15 mL

Chromogen substrate and counterstaining (depending on detection system)

●AEC substrate and AEC buffer	R007R.0000 R010R.0000	2 mL 15 mL
REMBRANDT® Methyl green counterstain	R016R.0000	15 mL
●NBT/BCIP	R008R.0000	15 mL
REMBRANDT® Nuclear fast red counterstain	R015R.0000	15 mL
REMBRANDT® Pepsin powder	R011R.0000	1 g
REMBRANDT® Pepsin diluent	R018R.0000	15 ml
REMBRANDT® Positive control slides, paraffin-embedded	Q300C.0000	2 pcs

Assay procedure

REMBRANDT® EBER RISH detection assay procedure for cytological specimen, frozen sections and FFPE tissue sections.

I. *Cytological specimen:*
Deposit the cells on coated glass slides and air dry for 30 minutes. Fix the slides with a cross-linking fixative (e.g. 4% para-formaldehyde) for 10 minutes at room temperature and rinse with PBS.
FFPE tissue sections:
For a detailed description of sample preparation and pre-treatment see: 2.1 Specimen collection and 2.2 Specimen pre-treatment of the PanPath RISH Manual.

II. Incubate both test and control slides (Q300C.0000) in pre-heated proteolytic work solution (prepare according to section 1.9 of RISH manual) (R011R.000 + R018R.000) at 37 ˚C. Paraffin-embedded sections (2.50 mg/ml), cytological specimen (100 µg/ml) or frozen sections (50 µg/ml) for 15 minutes.

III. Flush wash slides in deionised water, followed by dehydration in graded ethanol series (ethanol 70%, 96%, 96%, 100%, 100%) 1 minute each and air-dry slides for 15 minutes.

Do not treat more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete pre-treatment. Additionally, allow the slides to air-dry as recommended; otherwise sections will be lost.

IV. Homogenize probe solutions (A500P.XXXX) and spin briefly. Apply 1 drop of probe solution to each specimen (including control specimen (Q300C.0000)). Cover all specimens with a cover slip (avoid air bubbles).

V. Transfer the slides into a moist environment and incubate for 16 hours at 37 ˚C.

VI. Remove coverslips by soaking the slides in TBS buffer solution at room temperature.

VII. Take the slides out, wipe off excess buffer and dry the edges using a lint-free cloth.

VIII. Apply 2-3 drops of the appropriate conjugate (R0XXR.0000) to each specimen and transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate for 30 minutes.

In case of HRP detection, prepare AEC reaction mixture according to section 2.4 of the RISH manual.

IX. Tap off excess detection reagent and rinse the slides in TBS buffer at room temperature.

X. Transfer the slides into a container with deionised water and soak for 1 minute.

XI. Tap off excess water and dry around the edges using a lint-free cloth. Ensure that the specimen on the slide is not disrupted.

XII. Apply 2-3 drops of the approriate chromogen substrate reaction mixture (according to detection system, see RISH manual section 2.4) to each specimen. Transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate in the dark for 5-15 minutes.

XIII. Tap off excess substrate solution and rinse slides 3 times for 1 minute in fresh deionised water. The slides are now ready to be mounted or counterstained.

When a contrast colour is desired, the slides can be counterstained. If not, proceed to step XVI

XIV. Wipe off excess reagent and apply 2-3 drops of approriate counterstain to each specimen (see RISH manual section 2.5). Incubate for

at least 1 minute (longer incubation is possible and will yield in stronger staining).

XV. Tap off excess counterstain and rinse the slides briefly in deionised water.

XVI. Mount the slides by using an aqueous mounting medium. Interpret the results under a brightfield microscope.

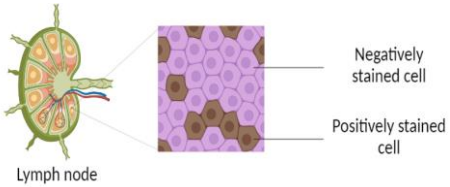
Interpretation of results

Hybridization of the REMBRANDT® EBER RISH probe is viewed using a brightfield microscope. The detection of the EBER1 and EBER2 miRNA is conducted by microscopic examination of cells. The stained cells that contain EBER1 and EBER2 miRNA sequences, stand out brightly against the de-stained cells. The REMBRANDT® EBER RISH detection assay procedure enables visual detection of EBV infected cells. Each signal corresponds with EBER miRNA. First, check if the positive control shows colour precipitations in conformity with the localisation of the target RNA. In the test slides, start under low power magnification and focus on localisation and colour to see whether:

•The positivity (colour precipitation) is observed in conformity with the localisation of the target RNA.
•The colour has the right shade (no endogenous or formalin pigment)

Use high power magnification to see whether:

•The positive staining texture (granular, etc), demarcation and localisation are conform the positive control staining pattern



For additional signal interpretation see RISH manual section 3.1.

Limitations of Procedure

i) The REMBRANDT® EBER RISH detection assay is solely applicable for the detection of EBER1 and EBER2 which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples or FFPE tissue sections).

ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. In tissue sections are required, the sections should be prepared in a 4 µm thickness. Furthermore, the tissues should be glued to the glass slides with a bio-adhesive (e.g. organ silane), dried at room temperature, subsequently dried at 37 °C overnight and lastly completely deparaffinized in xylene and alcohol series and air dried.

iii) Cytological specimen should be prepared as required by the user, fixed with cytological fixation agent, rinsed in distilled water prior to the ISH procedure and air dried.

iv) Many factors can influence the performance of the ISH procedure. Failure in detection can be due to i.e. improper sampling, handling, the time lapse between tissue removal and fixation, the size of the tissue specimen in the fixation medium, the fixation time, processing fixed tissue, the thickness of the section, the bio-adhesive on the slide, deparaffinisation procedure, incubation times, proteolytic pre-treatment, detection reagents, incubation temperatures and interpretation of results.

v) The performance of the RISH procedure is also affected by the sensitivity of the method and the presence of EBER1 and EBER2 miRNA. In case the limit of the sensitivity is reached a false negative reaction may be the result.

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viii) Therapeutic considerations based on the result of this test alone should not been taken. Results should be verified by other traditional diagnostic methods such as but not limited to clinical history, symptoms, as well as morphological data.

ix) The medical profession should be aware of risks and factors influencing the chromogenic signal intensity while interpretating the test result. Microscopy settings might influence the signal intensity and/or interpretation.

x) Laboratory personnel performing the test should be trained and knowledgeable to be able to interpret the test results.

Storage and handling

Store kit and its contents at 2-8°C. Store the dissolved and aliquoted reagents at recommended temperatures. When used and stored as indicated, the kit is stable until the expiry date printed on the box.

Product	Product number	Storage conditions
REMBRANDT® EBER RISH probe BIO or DIG detection	XXXP.XX XX	2-8 ˚C
REMBRANDT® Pepsin powder	R011R.00 00	Powder: 2-25˚C, ambient temperature

		Dissolved: -20˚C
REMBRANDT® Pepsin diluent	R018R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
REMBRANDT® Conjugated antibodies	R0XXR.0000	2-8 ˚C
REMBRANDT® TBS buffer pouch	R017R.0000	Powder: 2-25 ˚C Dissolved: 2-25 ˚C
REMBRANDT® NBT/BCIP substrate	R008R.0000	2-8 ˚C
REMBRANDT® AEC substrate	R007R.0000	2-8 ˚C
REMBRANDT® AEC buffer	R010R.0000	2-8 ˚C
REMBRANDT® Nuclear fast red counterstain	R015R.0000	2-8 ˚C
REMBRANDT® Methyl green counterstain	R016R.0000	2-8 ˚C
Positive control slides, paraffin embedded	Q300C.0000	2-25 ˚C

Hazard statements

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H351 - Suspected of causing cancer
H360D - May damage the unborn child
H373 - May cause damage to organs through prolonged or repeated exposure

Precautionary Statements

P202 - Do not handle until all safety precautions have been read and understood
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352 - IF ON SKIN: Wash with plenty of water and soap
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P362 + P364 - Take off contaminated clothing and wash it before reuse
P405 - Store locked up

Additional information

Product in combination with other devices

The REMBRANDT® RNA probes are intended for stand-alone usage. The assay is intended to be used in combination with standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), proteolytic-, detection- and other reagents (not supplied with this reagent) and a microscope. The combination has been tested and validated. Since the standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature controls, incubation time control(s) and other not supplied reagents such as but not limited to proteolytic reagents, detection reagents and a microscope is not combined with the device as a product, conformity with the essential requirements is not applicable. Assay validation criteria are mentioned in *'Interpretation of the Results'* and are also depending on clinical state of the sample, which may influence the validation criteria.

For additional information regarding the REMBRANDT® assays, a manual is included which specifies the following subjects:

- Controls
- Materials required but not included
- Storage and shelf-life
- Performance precautions
- Preparations of reagents
- Specimen collection
- Quality control
- Trouble shooting guide

Technical assistance

For technical assistance regarding the products performance, please contact info@panpath.nl or call +31 495499090. Visit our website for reprints of datasheets or additional documentation. www.panpath.nl

Literature list

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Disclaimer: This document is valid until the product expiry on the kit label