DATA SHEET-V1
REMBRANDT® 9P21 LOH FISH
DETECTION RESEARCH USE ONLY (RUO)

RUO

PanPath

Ref		
C810K.2030.05	Σ	5 T
C810K.2030.10	Σ	10 T

Intended use

I. The REMBRANDT® 9p21 LOH FISH detection assay is an assay intended for the detection of the human 9p21 region compared to the centromeric region of chromosome 9 by means of *in situ* hybridization.

II. The REMBRANDT® 9p21 LOH FISH detection assay is intended for the detection of the 9p21 locus compared to the centromeric region of chromosome 9 in fixed cells. The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis should not be taken on the basis of this test result.

III. The REMBRANDT® 9p21 LOH FISH detection assay kit is a quantitative assay for the detection of the 9p21 locus compared to the centromeric region of chromosome 9.

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

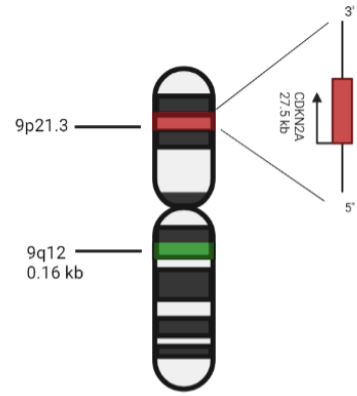
Clinical relevance

The LSI 9p21 probe is designed to target the 9p21 region (CDKN2A gene) within the 9p-arm. The cyclin-dependent kinase inhibitor 2A (CDKN2A) gene is a tumor suppressor gene, which has been shown to be deleted in a wide range of malignancies. Loss of the CDKN2A gene can result in cellular proliferation and proapoptotic pathways. CDKN2A produces two proteins: $p16^{INK4a}$ and $p14^{ARF}$, both of which have been linked to two tumor suppressor pathways: the RB and the p53 pathway. The mechanism of action involves binding to and inactivating of the cyclin D-cyclin-dependent kinase 4 complex, and thus rendering the retinoblastoma protein inactive. This results in blocking of the transcription of cell-cycle regulatory proteins and results in cell-cycle arrest (Liggett & Sidransky, 2016). In literature, deletion of 9p are frequently reported in patients with acute lymphoblastic leukemia, in B-cell acute lymphoblastic leukemia, 9p deletions are linked to disease progression (D'Angelo et al., 2008). Other diseases can be diffuse large B-cell lymphoma (Jardin et al., 2010) and glioma and meningiomas (Frazão et al., 2018). Lastly, losses of 9p have also been reported in malignant mesothelioma, melanoma (Yang et al., 2010) and bladder cancer (Knowles & Hurst, 2014) .

The LSI 9p21 LOH FISH detection assay is designed to target the CDKN2A gene compared to a CEP9 control probe which allows the detection of a deletion.

Probe specification

The REMBRANDT® 9p21 LOH FISH probe mix consists of a dsDNA probe detecting the 9p21 locus and a dsDNA centromeric probe detecting the centromeric region of chromosome 9. The centromeric region is detected by green fluorescence (AF488) and the locus is detected by orange fluorescent detection (AF555).
The REMBRANDT® 9p21 LOH FISH probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.



Test principle

In a fluorescent *in situ* hybridization assay, a double stranded DNA probe labelled with a fluorochrome is used. The labelled DNA probe is diluted in a hybridization mixture. The hybridization mixture containing the DNA probe is added to the specimen. A co-denaturation will ensure that the genomic DNA and the probe DNA become single stranded. After denaturation, the probe DNA is able to hybridize to its complementary target sequences in the cells. In the REMBRANDT® 9p21 LOH FISH detection assay, the fluorochrome is attached to the probe and the signals can be visualized directly by fluorescent microscopy after hybridization.

Reagents provided

Product name	Product number	Amount
Labelled probes	(depending on size choice)	

REMBRANDT® 9p21 LOH/CEP9-FISH probe mix	C810P.2030.05 or C810P.2030.10	Σ 5 T Σ 10 T
REMBRANDT® Pepsin powder	R011R.0000	1 g
REMBRANDT® Pepsin diluent	R018R.0000	15 ml
REMBRANDT® PanWash 4, 25X SSC	R025R.0000	4x 15 ml
REMBRANDT® Fluorescent Mounting medium	Z000R.0050	1 ml

Assay procedure

REMBRANDT® 9p21 LOH FISH detection assay procedure for cytological specimen.

I. Incubate slides in pre-heated proteolytic work solution (prepare according to section 1.9 of Manual-FISH) (R011R.000 + R018R.000) at 37 ˚C (100 µg/ml) for 15 minutes followed by a brief rinsing in 0.01M HCl (1x 2 minutes) and subsequent rinses in PBS (2x 1 minute).

II. 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.

III. Homogenize probe solution (C810P.2030.YY) a spin briefly. Apply 15 µl of probe solution to each specimen. Cover all specimens with a cover slip (avoid air bubbles). Denature slides on a 80 ˚C hotplate or other heating device for 3 minutes

Work in a pre-set order to ensure that all slides have been incubated at 80 ˚C for the exact same time. Do not denature more than 5 slides at the same time, the temperature of the heating device may drop dramatically, thus causing incomplete denaturation.

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

V. Remove coverslips by soaking the slides in PBS at room temperature

VI. Incubate the slides in diluted, pre-heated PanWash 4 (R025R.000) (prepare according to section 1.9 of Manual-FISH)

Do not incubate more than 5 slides at the same time in PanWash 4, the temperature of the PanWash 4 may drop dramatically, causing wrong stringency conditions.

VII. Incubate the slides in PBS at room temperature for 1 minute

VIII. Dehydrate the slides in graded ethanol series (70%, 96%, 96%, 100%, 100%) 1 minute each and air-dry the slides for 15 minutes (in the dark)

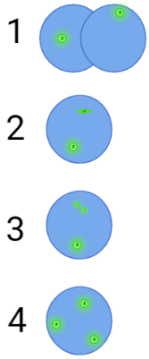
Mount the slides by applying mounting medium (Z000R.0050) and coverslip

Interpretation of results

Hybridization of the REMBRANDT® 9p21 LOH probe is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters. For green detection: λ_{exc} 492 nm, λ_{em} 517 nm and for orange detection: λ_{exc} 555 nm, λ_{em} 572 nm. Allowing visualization of orange fluorescent signal concentrated at the 9p21 locus, the green fluorescent signals concentrated at the centromeric region of chromosome 9 and the blue counterstained chromosomes and nuclei. The enumeration of the 9p21 locus is conducted by microscopic examination of interphase nuclei. The fluorescently-stained 9p21 locus stand out brightly against the general fluorescence of the nucleus. The 9p21 LOH procedure enables visual enumeration of copy numbers of the 9p21 locus within the nuclei. The assay results are reported as the percentage of nuclei with 0, 1, 2, 3, 4, and >4 fluorescent signals. Each fluorescent signal corresponds to a copy of the 9p21 locus or the centromeric region of chromosome 9.

Enumerate the fluorescent signals in the interphase nucleus using a 40X or 63X magnification. Objectives with higher magnification (eg, 63X or 100X) should be used to verify or resolve questions about split or diffused signals. Enumerate at least 100 nuclei per slide for accurate analysis.

•Two signals in close proximity and approximately the same sizes but not connected by a visible link are counted as 2 signals.
•Count a diffuse signal as 1 signal if diffusion of the signal is contiguous and within an acceptable boundary.
•Two small signals connected by a visible link are counted as 1 signal.
•Enumerate the number of nuclei with 0, 1, 2, 3, 4, or >4 signals. Count nuclei with 0 signals only if there are other nuclei with at least 1 signal present in the field of view. If the accuracy of the enumeration is in doubt, repeat the enumeration in another area of the slide.
•Do not enumerate nuclei with uncertain signals (Arsham et al., 2017)



Performance characteristics

Analytical Sensitivity and Specificity

The analytical sensitivity and specificity were investigated within PanPaths analytical performance assessment. Precision was investigated for the REMBRANDT® 9p21 LOH FISH detection assay and the results are available upon request.

Analytical sensitivity

The analytical sensitivity was determined in three levels. The normal cut-off percentage was determined based on assessment of 200 individual nuclei in two samples of healthy interphase lymphocytes from peripheral blood. The beta inversion was used to determine the percentage of normal-cut off. For the noise-to-signal percentage, the noise and signal values were determined for 100 signals in interphase lymphocytes from peripheral blood of two independent samples. The differences between noise and signal were evaluated using a 95% confidence interval. For the hybridization efficiency, 200 individual nuclei were assessed for the presence of FISH signals.

Performance characteristic	Outcome
Normal cut-off percentage	10%
Noise-to-signal cut-off percentage	18%
Hybridization efficiency	98%

Analytical specificity

The analytical specificity was determined in two levels. The theoretical specificity was determined by sequencing analysis of the probe DNA and mapping on Hg38. The practical specificity was determined by assessing the hybridization pattern in metaphase chromosomes of interphase lymphocytes from peripheral blood.

Performance characteristic	Outcome
Theoretical specificity	Mapped on chromosome 9, 9p21.3 and 9q12
Practical specificity	100%

Clinical performance

The clinical performance was not determined for the REMBRANDT® 9p21 LOH FISH detection assays since the assays do not detect a specific condition. The clinical performance is demonstrated by scientific validity studies.

Limitations of Procedure

i) The REMBRANDT® 9p21 LOH FISH detection assay is solely applicable for the detection of the 9p21 locus and the centromeric region of chromosome 9, which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples).

ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. If 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 ISH procedure is also affected by the sensitivity of the method and the presence of the 9p21 locus and centromeric region of chromosome 9. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® 9p21 LOH FISH 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. WGS).

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 fluorescent 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® 9p21 LOH/CEP9-FISH probe mix	C810P.XXXX	2-8 ˚C
REMBRANDT® Pepsin powder	R011R.0000	Powder: 2-25˚C, ambient temperature Dissolved: -20˚C
REMBRANDT® Pepsin diluent	R018R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
REMBRANDT® PanWash 4, 25X SSC	R025R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
REMBRANDT® Fluorescent mounting medium	Z000R.0050	2-8 ˚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® DNA 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

Arsham, M. S., Barch, M. J., & Lawce, H. J. (2017). *The AGT Cytogenetics Laboratory Manual The AGT Cytogenetics Laboratory Manual Edited by* (Vol. 4).

D'Angelo, G., Hotz, A. M., & Todeschin, P. (2008). Acute lymphoblastic leukemia with hypereosinophilia and 9p21 deletion: Case report and review of the literature. *Laboratory Hematology*, *14*(1), 7–9. https://doi.org/10.1532/LH96.07018

Frazão, L., Do Carmo Martins, M., Nunes, V. M., Pimentel, J., Faria, C., Miguéns, J., Sagarribay, A., Matos, M., Salgado, D., Nunes, S., Mafra, M., & Roque, L. (2018). BRAF V600E mutation and 9p21: CDKN2A/B and MTAP co-deletions - Markers in the clinical stratification of pediatric gliomas. *BMC*

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Jardin, F., Jais, J. P., Molina, T. J., Parmentier, F., Picquenot, J. M., Ruminy, P., Tilly, H., Bastard, C., Salles, G. A., Feugier, P., Thieblemont, C., Gisselbrecht, C., De Reynies, A., Coiffier, B., Haioun, C., & Leroy, K. (2010). Diffuse large B-cell lymphomas with CDKN2A deletion have a distinct gene expression signature and a poor prognosis under R-CHOP treatment: a GELA study. *Blood*, *116*(7), 1092–1104. https://doi.org/10.1182/BLOOD-2009-10-247122

Knowles, M. A., & Hurst, C. D. (2014). Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nature Reviews Cancer 2015 15:1*, *15*(1), 25–41. https://doi.org/10.1038/nrc3817

Liggett, W. H., & Sidransky, D. (2016). Role of the p16 tumor suppressor gene in cancer. *Https://Doi.Org/10.1200/JCO.1998.16.3.1197*, *16*(3), 1197–1206. https://doi.org/10.1200/JCO.1998.16.3.1197

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

DATA SHEET-V1
REMBRANDT® 9P21 LOH ISH
DETECTION RESEARCH USE ONLY (RUO)

RUO

PanPath

Ref

C810K.0199.05	Σ	5 T
C810K.0199.10	Σ	10 T

Intended use

I. The REMBRANDT® 9p21 LOH ISH detection assay is an assay intended for the detection of the human 9p21 region compared to the centromeric region of chromosome 9 by means of *in situ* hybridization.

II. The REMBRANDT® 9p21 LOH ISH detection assay is intended for the detection of the the 9p21 locus compared to the centromeric region of chromosome 9 in fixed cells. The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis should not be taken on the basis of this test result.

III. The REMBRANDT® 9p21 LOH ISH detection assay kit is a quantitative assay for the detection of the 9p21 locus compared to the centromeric region of chromosome 9.

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

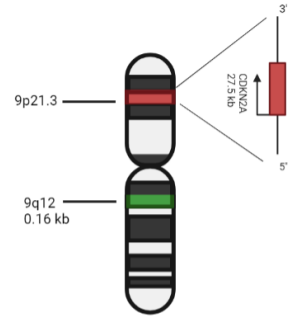
Clinical relevance

The LSI 9p21 probe is designed to target the 9p21 region (CDKN2A gene) within the 9p-arm. The cyclin-dependent kinase inhibitor 2A (CDKN2A) gene is a tumor suppressor gene, which has been shown to be deleted in a wide range of malignancies. Loss of the CDKN2A gene can result in cellular proliferation and proapoptotic pathways. CDKN2A produces two proteins: $p16^{INK4a}$ and $p14^{ARF}$, both of which have been linked to two tumor suppressor pathways: the RB and the p53 pathway. The mechanism of action involves binding to and inactivating of the cyclin D-cyclin-dependent kinase 4 complex, and thus rendering the retinoblastoma protein inactive. This results in blocking of the transcription of cell-cycle regulatory proteins and results in cell-cycle arrest (Liggett & Sidransky, 2016). In literature, deletion of 9p are frequently reported in patients with acute lymphoblastic leukemia, in B-cell acute lymphoblastic leukemia, 9p deletions are linked to disease progression (D'Angelo et al., 2008). Other diseases can be diffuse large B-cell lymphoma (Jardin et al., 2010) and glioma and meningiomas (Frazão et al., 2018). Lastly, losses of 9p have also been reported in malignant mesothelioma, melanoma (Yang et al., 2010) and bladder cancer (Knowles & Hurst, 2014) .

The LSI 9p21 LOH ISH detection assay is designed to target the CDKN2A gene compared to a CEP9 control probe which allows the detection of a deletion

Probe specification

The REMBRANDT® 9p21 LOH ISH probe mix consists of a dsDNA probe detecting the 9p21 locus and a dsDNA centromeric probe detecting the centromeric region of chromosome 9. The centromeric region is conjugated to biotin and the locus is conjugated to digoxigenin.
The REMBRANDT® 9p21 LOH ISH probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.



Test principle

In an *in situ* hybridization assay, a double stranded DNA probe labelled with a hapten is used. The labelled DNA probe is diluted in a hybridization mixture. The hybridization mixture containing the DNA probe is added to the specimen. A co-denaturation will ensure that the genomic DNA and the probe DNA become single stranded. After denaturation, the probe DNA is able to hybridize to its complementary target sequences in the cells. In the REMBRANDT® 9p21 LOH ISH detection assay, the haptens are attached to the probe and the signals can be visualized after detection by corresponding antibodies by fluorescent or brightfield microscopy

Reagents provided

Product name	Product number	Amount
Labelled probes (depending on size choice)		
REMBRANDT® LSI 9p21/CEP9-ISH probe mix	C810P.0199.05 or C810P.0199.10	Σ 5 T Σ 10 T

REMBRANDT® Pepsin powder	R011R.0000	1 g
REMBRANDT® Pepsin diluent	R018R.0000	15 ml
REMBRANDT® PanWash 4, 25X SSC	R025R.0000	4x 15 ml
REMBRANDT® Pre-treatment buffer	R026R.0000	15 ml
REMBRANDT® Fluorescent Mounting medium	Z000R.0050	1 ml

Assay procedure

REMBRANDT® 9p21 LOH ISH detection assay procedure for cytological specimen and FFPE tissue sections.

I. Specimen collection: for a detailed description of the specimen collection for cytological specimen or FFPE tissue sections see section 2.1 Specimen collection of the Manual ISH.

II. For FFPE tissue sections, after dewaxing, place slides in jar with pre-treatment solution (R026R.0000) in microwave set at i.e. 900W and incubate up until boiling. Subsequently, reset microwave at 180W and incubate for 10 minutes, followed by cooling down for 20 minutes at room temperature. Flush wash slides in deionised water.

III. Incubate slides in pre-heated proteolytic work solution (prepare according to section 1.9 and 1.10 of Manual ISH (R011R.000 + R018R.000) at 37 ˚C. Paraffin-embedded sections (1.25 mg/ml) or cytological specimen (100 µg/ml) for 15 minutes.

IV. 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.

V. Homogenize probe solution (C810P.0199.YY) and spin briefly. Apply 10-15 µl of probe solution to each specimen. Cover all specimens with a cover slip (avoid air bubbles). Denature slides on a 80 ˚C hotplate or other heating device, 3 minutes for cytological specimen and 10 minutes for FFPE tissue sections.

Work in a pre-set order to ensure that all slides have been incubated at 80 ˚C for the exact same time. Do not denature more than 5 slides at the same time, the temperature of the heating device may drop dramatically, thus causing incomplete denaturation.

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

VII. Remove coverslips by soaking the slides in PBS at room temperature.

VIII. Incubate the slides in diluted, pre-heated PanWash 4 (R025R.0000) (prepare according to section 1.9 of Manual-ISH). For cytological specimen and FFPE tissue sections, 2x 5 minutes in 2x SSC at 42 ˚C. For cytological specimen, subsequently incubate 2x 5 minutes in 0.1x SSC at 61 ˚C.

Do not incubate more than 5 slides at the same time in PanWash 4, the temperature of the PanWash 4 may drop dramatically, causing wrong stringency conditions.

IX. Appropriate detection system should be evaluated by the end-user. Recommended detection systems are listed below

Digoxigenin detection	Biotin detection
R003R.0000 REMBRANDT® Sheep aDig-AP conjugate	R041R.0000 REMBRANDT® Goat aBio-AP Fab conjugate
R004R.0000 REMBRANDT® Sheep aDig-HRP conjugate	R042R.0000 REMBRANDT® Goat aBio-HRP Fab conjugate

AP detection	HRP detection
R008R.0000 REMBRANDT® NBT/BCIP substrate	R007R.0000 REMBRANDT® AEC substrate + R010R.0000 REMBRANDT® AEC buffer

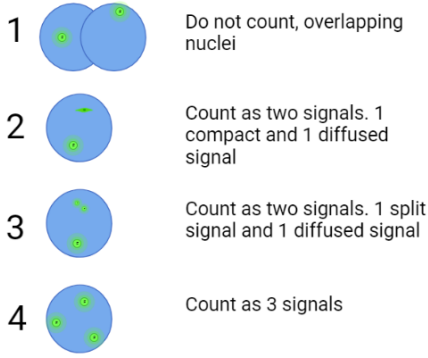
Interpretation of results

Hybridization of the REMBRANDT® 9p21 LOH detection assay is conducted by microscopic examination of interphase nuclei (fluorescence or brightfield, depending on antibodies used for detection). The fluorescently or chromogenic-stained 9p21 loci stand out brightly against the nucleus. The enumeration of the locus 9p21 is conducted by microscopic examination of interphase nuclei. The LSI 9p21 procedure enables visual enumeration of copy numbers of the 9p21 locus within the nuclei, with CEP9 as a control. The assay results are reported as the percentage of nuclei with 0, 1, 2, 3, 4, and >4 signals. Each signal corresponds to a copy of the 9p21 locus or the centromeric region of chromosome 9.

Enumerate the fluorescent signals in the interphase nucleus using a 40X or 63X magnification. Objectives with

higher magnification (eg, 63X or 100X) should be used to verify or resolve questions about split or diffused signals. Enumerate at least 100 nuclei per slide for accurate analysis.

•Two signals in close proximity and approximately the same sizes but not connected by a visible link are counted as 2 signals.
•Count a diffuse signal as 1 signal if diffusion of the signal is contiguous and within an acceptable boundary.
•Two small signals connected by a visible link are counted as 1 signal.
•Enumerate the number of nuclei with 0, 1, 2, 3, 4, or >4 signals. Count nuclei with 0 signals only if there are other nuclei with at least 1 signal present in the field of view. If the accuracy of the enumeration is in doubt, repeat the enumeration in another area of the slide.
•Do not enumerate nuclei with uncertain signals (Arsham et al., 2017)



Performance characteristics

Analytical Sensitivity and Specificity

The analytical sensitivity and specificity were investigated within PanPaths analytical performance assessment. Precision was investigated for the REMBRANDT® 9p21 LOH ISH detection assay and the results are available upon request.

Analytical sensitivity

The analytical sensitivity was determined in three levels. The normal cut-off percentage was determined based on assessment of 200 individual nuclei in two samples of healthy interphase lymphocytes from peripheral blood. The beta inversion was used to determine the percentage of normal-cut off. For the noise-to-signal percentage, the noise and signal values were determined for 100 signals in interphase lymphocytes from peripheral blood of two independent samples. The differences between noise and signal were evaluated using a 95% confidence interval. For the hybridization efficiency, 200 individual nuclei were assessed for the presence of ISH signals.

Performance characteristic	Outcome
Normal cut-off percentage	10%
Noise-to-signal cut-off percentage	18%
Hybridization efficiency	98%

Analytical specificity

The analytical specificity was determined in two levels. The theoretical specificity was determined by sequencing analysis of the probe DNA and mapping on Hg38. The practical specificity was determined by assessing the hybridization pattern in metaphase chromosomes of interphase lymphocytes from peripheral blood.

Performance characteristic	Outcome
Theoretical specificity	Mapped on chromosome 9, 9p21.3 and 9q12.
Practical specificity	100%

Clinical performance

The clinical performance was not determined for the REMBRANDT® 9p21 LOH ISH detection assays since the assays do not detect a specific condition. The clinical performance is demonstrated by scientific validity studies.

Limitations of Procedure

i) The REMBRANDT® 9p21 LOH FISH detection assay is solely applicable for the detection of the 9p21 locus and the centromeric region of chromosome 9, which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples).
ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. If 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 ISH procedure is also affected by the sensitivity of the method and the presence of the 9p21 locus and centromeric region of chromosome 9. In case the limit of the sensitivity is reached a false negative reaction may be the result.
vi) The REMBRANDT® 9p21 LOH FISH 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. WGS).
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 fluorescent 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® LSI 9p21/CEP9-ISH probe mix	C810P.XXXX	2-8 ˚C
REMBRANDT® Pepsin powder	R011R.0000	Powder: 2-25˚C, ambient temperature Dissolved: -20˚C
REMBRANDT® Pepsin diluent	R018R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
REMBRANDT® PanWash 4, 25X SSC	R025R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
REMBRANDT® Pre-treatment buffer	R026R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
REMBRANDT® Fluorescent mounting medium	Z000R.0050	2-8 ˚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® DNA 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

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

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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