DATA SHEET-V1
REMBRANDT® PRADER-WILLI/ANGELMAN SYNDROME (PWS/AS) FISH DETECTION

CE IVD

PanPath

Ref		
C804K.2030.05	Σ	5 T
C804K.2030.10	Σ	10 T

Intended use

I. The REMBRANDT® Prader-Willi/Angelman syndrome (PWS/AS) FISH detection assay is an *in-vitro* diagnostics medical device intended for the detection of a deletion in the PWS/AS critical region 15q11.2, in comparison to the centromeric region of the human chromosome 15, by means of *in situ* hybridization.

II. The REMBRANDT® Prader-Willi/Angelman syndrome (PWS/AS) FISH detection assay is intended for the detection of a deletion in the PWS/AS critical region 15q11.2, in comparison to the centromeric region of the human chromosome 15 in fixed cells. 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® Prader-Willi/Angelman syndrome (PWS/AS) FISH detection assay kit is a qualitative assay for the detection of a deletion in the PWS/AS critical region 15q11.2, in comparison to the centromeric region of the human chromosome 15.

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

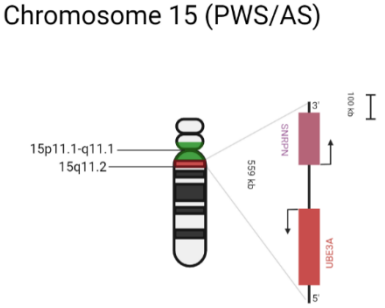
Clinical relevance

15q11.2 is a critical region for multiple microdeletion syndromes, such as the Prader-Willi syndrome (PWS) and the Angelman syndrome (AS). PWS and AS are distinct disorders caused by a heterozygous microdeletion in the 15q11.2 region (Zhang et al., 2021). This microdeletion occurs in about 1 in 10,000 births worldwide. 70% of PWS/AS cases are caused by a de novo microdeletion in the Prader-Willi/ Angelman critical region (PWACR) (G. Butler, 2011) .The remaining 30% is caused by a maternal uniparental disomy, meaning that both chromosome copies are received from the mother (Wang et al., 2004; Zhang et al., 2021).

The PWACR contains the UBE3A (ubiquitin protein ligase E3A) and SNRPN (small nuclear ribonucleoprotein polypeptide N) gene. The characteristic phenotype of PWS includes restricted growth, hypotonia and physical aggression. The characteristic phenotype of AS includes distinct happy facial features, hyperactivity, epilepsy and microcephaly . The severity of PWS/AS can be worsened by an unbalanced translocation of the PML gene on 15q22 (Smith et al., 2000). Rembrandt PWS/AS FISH probes are designed to target the UBE3A and the SNRPN gene, mapping the Prader-Willi/Angelman critical region (PWACR) of 559 kb long.

Probe specification

The REMBRANDT® Prader-Willi/Angelman probe set consists of a 559 kb probe detecting the 15q11.2 locus and a 3.5 kb centromeric probe detecting the centromeric region of chromosome 15. The centromeric region is detected by green fluorescence (AF488) and the locus is detected by orange fluorescent detection (AF555). The Prader-Willi/Angelman syndrome (PWS/AS) FISH detection assay is able to detect a deletion in the Prader-Willi/Angelman syndrome critical region 15q11.2, in comparison to the centromeric region of the human chromosome 15 by means of direct *in situ* hybridization. The Prader-Willi/Angelman 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® Prader-Willi/Angelman syndrome (PWS/AS) 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 probe (depending on label and size choice)		
●PWS/AS -FISH probes orange/ green detection	C804P.2030.05	Σ 5 T
●PWS/AS -FISH probes orange/ green detection	C804P.2030.10	Σ 10 T
Pepsin powder	R011R.0000	1 g
Pepsin diluent	R018R.0000	15 ml
PanWash 4, 25X SSC	R025R.0000	15 ml
Fluorescent Mounting medium	Z000R.0050	1 ml

Assay procedure

REMBRANDT® Prader-Willi/Angelman syndrome (PWS/AS) 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 (C804P.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)

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

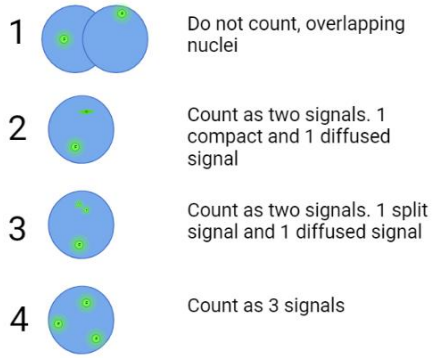
Interpretation of results

Hybridization of the PWS/AS probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters for orange detection: λ_{exc} 555 nm, λ_{em} 572 nm. Allowing visualization of orange fluorescent signal concentrated at the 15q11.2 locus of chromosome 15 in combination with green fluorescent signals representing the centromeric region of chromosome 15. The enumeration 15q11.2 copy numbers conducted by microscopic examination of interphase nuclei, compared to the signals representing chromosome 15. The fluorescently-stained locus of chromosome 15 stand out brightly against the general fluorescence of the nucleus.

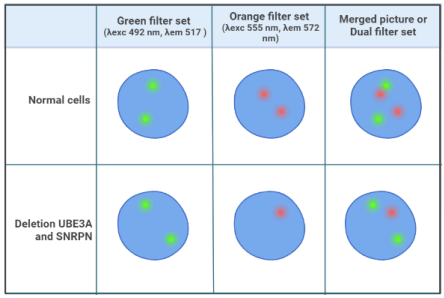
The Prader-Willi/Angelman syndrome (PWS/AS) FISH procedure enables observation of a possible detection of a deletion in the PWS/AS critical region 15q11.2, in comparison to the centromeric region of the human chromosome 15 within the nuclei. The assay results are reported as the percentage of nuclei with 0, 1, 2, 3, 4, and >4 fluorescent signals. Each orange fluorescent signal corresponds to the 15q11.2 locus, and each green fluorescent signal corresponds to the centromere of chromosome 15.

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 ('Marilyn S., 2017)



Expected results in normal and aberrant nuclei



Other signal distribution may be observed in some abnormal samples which might result in a different signal pattern than described above. Unexpected signal patterns should be further investigated.

Performance characteristics

Analytical Sensitivity and Specificity

The analytical sensitivity and specificity were investigated within PanPaths analytical performance assessment. Precision was investigated for the Prader-Willi/Angelman syndrome (PWS/AS) FISH detection assay and 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	9.8%
Noise-to-signal cut-off percentage	25.4%
Hybridization efficiency	99.5%

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 the locus 15q11.2 and the centromeric region 15p11.1-q11.1
Practical specificity	100%

Limitations of Procedure

i) The REMBRANDT® Prader-Willi/Angelman syndrome (PWS/AS) FISH detection assay is solely applicable detection of a deletion in the PWS/AS critical region 15q11.2, in comparison to the centromeric region of the human chromosome 15, 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. 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 ISH procedure is also affected by the sensitivity of the method and the presence of the centromeric region of chromosome 15. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® Prader-Willi/Angelman syndrome (PWS/AS) 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
15q11.2 CEP15 Probe orange /green detection	C804P.2030	2-8 ˚C
Pepsin powder	R011R.0000	Powder: 2-25˚C, ambient temperature Dissolved: -20˚C
Pepsin diluent	R018R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
PanWash 4, 25X SSC	R025R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
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 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

G. Butler, M. (2011). Prader-Willi Syndrome: Obesity due to Genomic Imprinting. *Current Genomics*, *12*(3), 204–215. https://doi.org/10.2174/138920211795677877

'Marilyn S., A. 'Margaret J. , B. ' H. J. , L. (2017). *The AGT Cytogenetics Laboratory Manual* (4th Edition).

Smith, A., Jauch, A., Heaps, L. S., Robson, L., & Kearney, B. (2000). Unbalanced translocation t(15;22) in 'severe' Prader–Willi syndrome. *Annales de Génétique*, *43*(3–4), 125–130. https://doi.org/10.1016/S0003-3995(00)01017-0

Wang, Y.-M., Chuang, L., Wang, B.-T., & Kuo, P.-L. (2004). Maternal uniparental disomy in a patient with Prader-Willi syndrome with an additional small inv dup(15) chromosome. *Journal of the Formosan Medical Association = Taiwan Yi Zhi*, *103*(12), 943–947.

Zhang, K., Liu, S., Gu, W., Lv, Y., Yu, H., Gao, M., Wang, D., Zhao, J., Li, X., Gai, Z., Zhao, S., Liu, Y., & Yuan, Y. (2021). Transmission of a Novel Imprinting Center Deletion Associated With Prader–Willi Syndrome Through Three Generations of a Chinese Family: Case Presentation, Differential Diagnosis, and a Lesson Worth Thinking About. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.630650

DATA SHEET-V1
REMBRANDT® LSI 15Q11.2/CEP15-FISH DETECTION
RESEARCH USE ONLY (RUO)

RUO

PanPath

Ref

C804K.2030.05	Σ	5 T
C804K.2030.10	Σ	10 T

Intended use

I. The REMBRANDT® LSI 15q11.2/CEP15-FISH assay is for research use only and is intended for the detection of the locus 15q11.2, in comparison to the centromeric region of the human chromosome 15 in fixed cells.

II. The REMBRANDT® LSI 15q11.2/CEP15-FISH assay kit is a qualitative assay for the detection of the locus 15q11.2, in comparison to the centromeric region of the human chromosome 15. A clinical diagnosis should not be established based on the performance of this test.

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

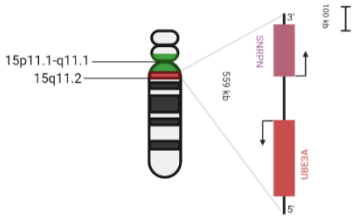
Clinical relevance

15q11.2 is a critical region for multiple microdeletion syndromes, such as the Prader-Willi syndrome (PWS) and the Angelman syndrome (AS). PWS and AS are distinct disorders caused by a heterozygous microdeletion in the 15q11.2 region (Zhang et al., 2021). This microdeletion occurs in about 1 in 10,000 births worldwide. 70% of PWS/AS cases are caused by a de novo microdeletion in the Prader-Willi/ Angelman critical region (PWACR) (G. Butler, 2011) .The remaining 30% is caused by a maternal uniparental disomy, meaning that both chromosome copies are received from the mother (Wang et al., 2004; Zhang et al., 2021).

The PWACR contains the UBE3A (ubiquitin protein ligase E3A) and SNRPN (small nuclear ribonucleoprotein polypeptide N) gene. The characteristic phenotype of PWS includes restricted growth, hypotonia and physical aggression. The characteristic phenotype of AS includes distinct happy facial features, hyperactivity, epilepsy and microcephaly . The severity of PWS/AS can be worsened by an unbalanced translocation of the PML gene on 15q22 (Smith et al., 2000). Rembrandt PWS/AS FISH probes are designed to target the UBE3A and the SNRPN gene, mapping the Prader-Willi/Angelman critical region (PWACR) of 559 kb long.

Probe specification

The REMBRANDT® LSI 15q11.2/CEP15 probe set consists of a 559 kb probe detecting the 15q11.2 locus and a 3.5 kb centromeric probe detecting the centromeric region of chromosome 15. The centromeric region is detected by green fluorescence (AF488) and the locus is detected by orange fluorescent detection (AF555). The Prader-Willi/Angelman syndrome (PWS/AS) FISH detection assay is able to detect a deletion in the Prader-Willi/Angelman syndrome critical region 15q11.2, in comparison to the centromeric region of the human chromosome 15 by means of direct *in situ* hybridization. The Prader-Willi/Angelman 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® LSI 15q11.2/CEP15-FISH 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 label and size choice)		
●LSI 15q11.2/CEP15 - FISH probes orange/green detection	C804P.2030.05	Σ 5 T
●LSI 15q11.2/CEP15 -	C804P.2030.10	Σ 10 T

FISH probes orange/green detection		
Pepsin powder	R011R.0000	1 g
Pepsin diluent	R018R.0000	15 ml
PanWash 4, 25X SSC	R025R.0000	15 ml
Fluorescent Mounting medium	Z000R.0050	1 ml

Assay procedure

REMBRANDT® LSI 15q11.2/CEP15-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 (C804P.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)

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

Interpretation of results

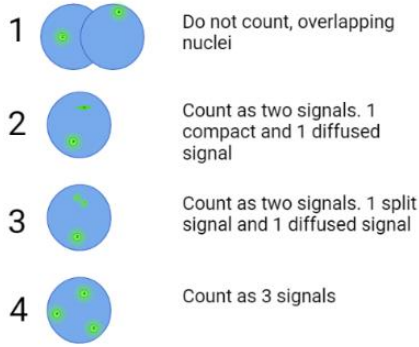
Hybridization of the LSI 15q11.2/CEP15 probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters for orange detection: λ_{exc} 555 nm, λ_{em} 572 nm. Allowing visualization of orange fluorescent signal concentrated at the 15q11.2 locus of chromosome 15 in combination with green fluorescent signals representing the centromeric region of chromosome 15. The enumeration 15q11.2 copy numbers conducted by microscopic examination of interphase nuclei, compared to the signals representing chromosome 15. The fluorescently-stained locus of chromosome 15 stand out brightly against the general fluorescence of the nucleus.

The assay results are reported as the percentage of nuclei with 0, 1, 2, 3, 4, and >4 fluorescent signals. Each orange fluorescent signal corresponds to the 15q11.2 locus, and each green fluorescent signal corresponds to the centromere of chromosome 15.

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 ('MarilynS., 2017)



Performance characteristics

Analytical Sensitivity and Specificity

The analytical sensitivity and specificity were investigated within PanPaths analytical performance assessment. Precision was investigated for the LSI 15q11.2/CEP15-FISH assay and 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	9.8%
Noise-to-signal cut-off percentage	25.4%
Hybridization efficiency	99.5%

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 the locus 15q11.2 and the centromeric region 15p11.1-q11.1
Practical specificity	100%

Limitations of Procedure

i) The LSI 15q11.2/CEP15 FISH assay is solely applicable detection the 15q11.2 locus, in comparison to the centromeric region of the human chromosome 15, 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. 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 ISH procedure is also affected by the sensitivity of the method and the presence of the 15q11.2 locus and the centromeric region of chromosome 15. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The LSI 15q11.2/CEP15-ISH 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 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.

viii) 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
15q11.2/CEP15 Probe orange /green detection	C804P.2030	2-8 ˚C

Pepsin powder	R011R.0000	Powder: 2-25˚C, ambient temperature Dissolved: -20˚C
Pepsin diluent	R018R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
PanWash 4, 25X SSC	R025R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
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
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Additional information

Product in combination with other devices

The REMBRANDT® DNA 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

G. Butler, M. (2011). Prader-Willi Syndrome: Obesity due to Genomic Imprinting. *Current Genomics*, *12*(3), 204–215. https://doi.org/10.2174/138920211795677877

'Marilyn S., A. 'Margaret J. , B. ' H. J. , L. (2017). *The AGT Cytogenetics Laboratory Manual* (4th Edition).

Smith, A., Jauch, A., Heaps, L. S., Robson, L., & Kearney, B. (2000). Unbalanced translocation t(15;22) in 'severe' Prader–Willi syndrome. *Annales de Génétique*, *43*(3–4), 125–130. https://doi.org/10.1016/S0003-3995(00)01017-0

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DATA SHEET-V1
REMBRANDT® LSI 15Q11.21/CEP15-ISH DETECTION
RESEARCH USE ONLY (RUO)

RUO

PanPath

Ref

C804K.0199.05	Σ	5 T
C804K.0199.10	Σ	10 T

Intended use

I. The REMBRANDT® LSI 15q11.2/CEP15-ISH detection assay is an in-vitro diagnostics medical device intended for the detection of the 15q11.2 locus, in comparison to the centromeric region of the human chromosome 15, by means of *in situ* hybridization.

II. The REMBRANDT® LSI 15q11.2/CEP15-ISH detection assay is intended for the detection the 15q11.2 locus, in comparison to the centromeric region of the human chromosome 15 in fixed cells. A clinical diagnosis should not be established based on the performance of this test.

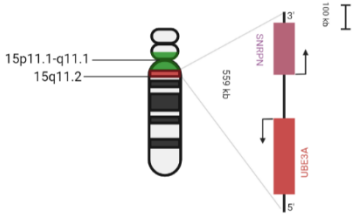
III. The intended users are qualified laboratory employees in cytology and/or pathology. The product is intended for professional use.

Clinical relevance

15q11.2 is a critical region for multiple microdeletion syndromes, such as the Prader-Willi syndrome (PWS) and the Angelman syndrome (AS). PWS and AS are distinct disorders caused by a heterozygous microdeletion in the 15q11.2 region (Zhang et al., 2021). This microdeletion occurs in about 1 in 10,000 births worldwide. 70% of PWS/AS cases are caused by a de novo microdeletion in the Prader-Willi/ Angelman critical region (PWACR) (G. Butler, 2011) .The remaining 30% is caused by a maternal uniparental disomy, meaning that both chromosome copies are received from the mother (Wang et al., 2004; Zhang et al., 2021). The PWACR contains the UBE3A (ubiquitin protein ligase E3A) and SNRPN (small nuclear ribonucleoprotein polypeptide N) gene. The characteristic phenotype of PWS includes restricted growth, hypotonia and physical aggression. The characteristic phenotype of AS includes distinct happy facial features, hyperactivity, epilepsy and microcephaly . The severity of PWS/AS can be worsened by an unbalanced translocation of the PML gene on 15q22 (Smith et al., 2000). Rembrandt PWS/AS FISH probes are designed to target the UBE3A and the SNRPN gene, mapping the Prader-Willi/Angelman critical region (PWACR) of 559 kb long.

Probe specification

The REMBRANDT® LSI 15q11.2/CEP15 probe set consists of a 559 kb probe detecting the 15q11.2 locus and a 3.5 kb centromeric probe detecting the centromeric region of chromosome 15. The centromeric region is conjugated to biotin and the locus is conjugated to digoxigenin. The LSI 15q11.2/CEP15-ISH assay is able to detect the 15q11.2 locus, in comparison to the centromeric region of the human chromosome 15 by means of direct *in situ* hybridization. The LSI 15q11.2/CEP15 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® LSI 15q11.2/CEP15-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 label and size choice)		
●LSI 15q11.2/CEP15-ISH probes biotin/digoxigenin detection	C804P.0199.05	Σ 5 T
●LSI 15q111.2/CEP15-ISH probes biotin/digoxigenin detection	C804P.0199.10	Σ 10 T
Pepsin powder	R011R.0000	1 g
Pepsin diluent	R018R.0000	15 ml
PanWash 4, 25X SSC	R025R.0000	15 ml

Assay procedure

REMBRANDT® Prader-Willi/Angelman syndrome (PWS/AS) ISH 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 (C804P.0199.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. Appropriate detection system should be evaluated by the end-user. Recommended detection systems are listed below

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

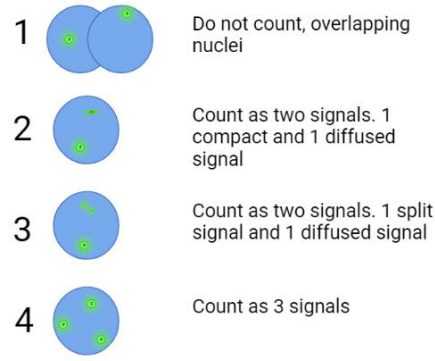
Interpretation of results

Hybridization of the LSI 15q11.2/CEP15 probes is viewed using a fluorescence of brightfield microscope equipped with appropriate excitation and emission filters. Allowing visualization of a signal concentrated at the 15q11.2 locus of chromosome 15 in combination with signals representing the centromeric region of chromosome 15. The enumeration 15q11.2 copy numbers conducted by microscopic examination of interphase nuclei, compared to the signals representing chromosome 15.

The assay results are reported as the percentage of nuclei with 0, 1, 2, 3, 4, and >4 signals.

Enumerate the 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



Performance characteristics

The REMBRANDT® LSI 15q11.2/CEP15-ISH detection assay was analytically validated for LSI 15q11.2/CEP15-FISH detection. The results of the direct fluorescent assay are shown. However, for the REMBRANDT® LSI 15q11.2/CEP15-ISH detection assay, the detection system may influence the performance characteristics and LSI 15q11.2/CEP15-ISH detection assay in combination with different detection systems should be evaluated carefully by the end-user.

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	9.8%
Noise-to-signal cut-off percentage	25.4%
Hybridization efficiency	99.5%

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 15, q11.2 and the centromeric region p11.1-q11.1
Practical specificity	100%

Limitations of Procedure

i) The LSI 15q11.2/CEP15-ISH detection assay is solely applicable for the detection of the 15q11.2 locus, in comparison to the centromeric region of the human chromosome 15, 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. 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 ISH procedure is also affected by the sensitivity of the method and the presence of the 15q11.2 locus and the centromeric region of chromosome 15. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The LSI 15q11.2/CEP15-ISH 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.

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
LSI 15q11.2/CEP15-ISH probes biotin/digoxigenin detection	C804P.0199	2-8 ˚C
PanWash 4, 25X SSC	R025R.0000	Concentrated solution and diluted: 2-25˚C, ambient temperature
Pepsin powder	R011R.0000	Powder: 2-25˚C,

		ambient temperature
Pepsin diluent	R018R.0000	Dissolved: -20˚C Concentrated solution and diluted: 2-25˚C, ambient temperature

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
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