DATA SHEET-V2
REMBRANDT® PRENATAL ENUMERATION FISH DETECTION ASSAY RESEARCH USE ONLY (RUO)

RUO

Pan Path

Ref

C818K.203040.05	Σ	5 T
C818K.203040.10	Σ	10 T

Intended use

I. The REMBRANDT® Prenatal Enumeration FISH detection assay is an assay intended for the detection of an imbalance of the human 13q12 and 21q22 loci or the centromeric region of chromosome X, Y and 18 relative to each other by means of *in situ* hybridization.

II. The REMBRANDT® Prenatal Enumeration FISH detection assay is intended for the detection of an imbalance of the human 13q12 or 21q22 loci or the centromeric region of chromosome X, Y and 18 relative to each other 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® Prenatal Enumeration FISH detection assay kit is a quantitative assay for the detection of an imbalance of the human 13q12 and 21q22 loci or the centromeric region of chromosome X, Y and 18 relative to each other.

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

Clinical relevance

The REMBRANDT® Prenatal Enumeration is designed for the detection of the most common fetal chromosomal disorders. These are Down syndrome (Trisomy 21), Edwards syndrome (Trisomy 18), the 13q12 microdeletion syndrome and the Patau syndrome (Trisomy 13); and sex chromosome disorders (consisting of aneuploidy of chromosome X and/or Y).

The Patau syndrome exists when three copies of chromosome 13 are present. It expresses itself with severe congenital abnormalities, including heart defects, brain malformations and cleft lip/palate, which can lead to significant intellectual disabilities (Kuznetsova et al., 2023).

The 13q12.3 microdeletion syndrome is a rare chromosomal anomaly characterized by intellectual disability, speech delay, postnatal microcephaly, eczema/atopic dermatitis
and characteristic facial features and reduced sensitivity to pain (Bartholdi et al., 2014).

The 13q12.3 locus corresponds to the HMGB1 gene, this is a gene that encodes a protein belonging to the High Mobility Group-box superfamily. The encoded non-histone, nuclear DNA-binding protein regulates transcription and is involved in the organization of DNA. The protein plays a role in several cellular processes such as inflammation, cell differentiation and tumor cell migration (National Library of Medicine, 2023).

The 21q22.13 locus corresponds with the *DSCR4 (Down Syndrome Critical Region 4), DSCR8 (Down Syndrome Critical Region 8) and DSCR10 (Down Syndrome Critical Region 10)* genes. These genes can be associated with Down syndrome (Trisomy 21), which is caused by an extra copy of chromosome 21 . Down syndrome is characterized by developmental and intellectual disabilities, as well as various physical features (Pelleri et al., 2016). A probe that target these genes can be used to detect chromosomal abnormalities, which can identify abnormalities such as Down syndrome.
The LSI 21q22.13 FISH probes is designed to target the *DSCR4, DSCR8, DSCR10 and KCNJ15* genes.

The presence of three copies of chromosome 18, trisomy 18, results in the Edwards syndrome. The Edwards syndrome is the second most common autosomal trisomy discovered at birth, behind Down syndrome (chromosome 21 trisomy). The importance lies on the high prevalence of the syndrome, which is estimated between 1:3600 – 1:8500 live births in different areas of the world. In trisomy 18, the ratio of male to female is 1:2 (Crawford & Dearmun, 2016; Rosa et al., 2013). Babies born with Edwards syndrome mostly have life-threatening abnormalities and a poor prognosis. It is associated with structural abnormalities (such as ventricular and atrial septal defects and patent ductus arteriosus, gastrointestinal anomalies (such as esophageal atresia or exomphalos) and skeletal abnormalities (such as foot and hand malformations).

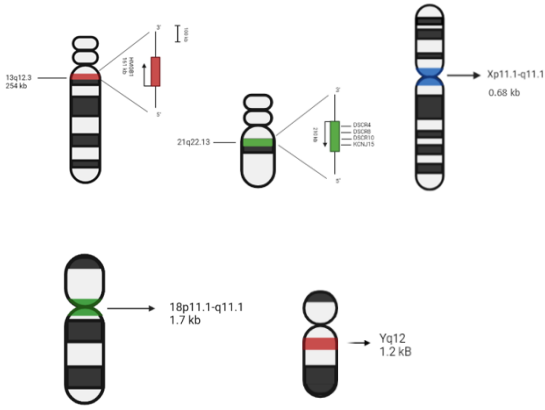
Aneuploidy involving the sex chromosomes X and Y often leads to distinct genetic conditions and health implications. Conditions like Klinefelter syndrome (47, XXY) and Turner syndrome (45, X), resulting from X chromosome aneuploidies, manifest with characteristic physical and development features, which can impact fertility, hormone regulation and can increase the risk of certain health issues such as cardiovascular problems (Skuse et al., 2018). Aneuploidies involving the Y chromosome, like XYY syndrome, are often less symptomatic but may still present with behavioral or developmental differences and require tailored support and intervention strategies (Visootsak et al., 2007).

Probe specification

The REMBRANDT® Prenatal Enumeration FISH detection consists of two hybridization mixtures.

- The first FISH probe mixture consists of a 254 kb dsDNA probe detecting the 13q12 locus and a 210 kb dsDNA probe detecting the 21q22.13 locus. The 13q12 locus is detected by green fluorescence (AF488) and the 21q22.13 locus is detected by orange detection (AF555).
- The second FISH probe mixture consists of a 0.68 kb dsDNA centromeric probe detecting the centromeric region of chromosome X, a 1.2 kb dsDNA centromeric probe detecting the centromeric region of chromosome Y and a 1.7 kb dsDNA probe detecting the centromeric region of chromosome 18. The centromeric region of chromosome X is detected by green fluorescence (AF488), the centromeric region of chromosome Y is detected by orange fluorescence (AF555) and the the centromeric region of chromosome 18 is detected by blue fluorescence (Atto425).

The REMBRANDT® Prenatal Enumeration FISH probes are ready-to-use solutions that have been pre-mixed in a hybridization mixture containing formamide, dextran sulfate and SSC.



Test principle

In a fluorescent *in situ* hybridization assay, a double-stranded DNA probe labeled with a fluorochrome is used. The labeled 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 patient DNA and the probe DNA become single-stranded. After denaturation, the probe DNA can hybridize to its complementary target sequences in the cells. In the REMBRANDT® Prenatal Enumeration 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
Labeled probes (depending on size choice)		
REMBRANDT® FISH probe mixes		
13q12/21q22 and CEPX/CEPY/CEP18	C818P.2030.1.05 C818P.203040.2.05	Σ 5 T
	or	
13q12/21q22 and CEPX/CEPY/CEP18	C818P.2030.1.10 C818P.203040.2.10	Σ 10 T
REMBRANDT® Pepsin powder	R011R.0000	1 g
REMBRANDT® Pepsin diluent	R018R.0000	15 ml
REMBRANDT® PanWash, 25X SSC	R025R.0000	4x 15 ml
REMBRANDT® Fluorescent Mounting medium	Z000R.0050	1 ml

Assay procedure

REMBRANDT® Prenatal Enumeration imbalance 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 solutions (C818P.203040.YY) and spin briefly. Apply 15 µl of probe solution to each specimen. Cover all specimens with a cover slip (avoid air bubbles). Denature slides on an 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 (R025R.000) (prepare according to section 1.9 of Manual-FISH)

Do not incubate more than 5 slides at the same time in PanWash, the temperature of the PanWash 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)

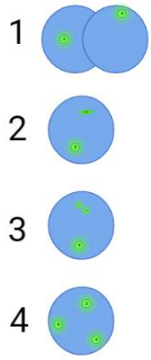
Mount the slides by applying mounting medium (Z000R.0050) and coverslip

Interpretation of results

Hybridization of the REMBRANDT® Prenatal Enumeration imbalance 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, for green detection: λ_{exc} 492 nm, λ_{em} 517 nm and for blue detection: λ_{exc} 436 nm, λ_{em} 478 nm. This allows the visualization of green fluorescent signals concentrated at the 13q12 loci and orange fluorescent signals at the 21q22.13 loci; green fluorescent signals concentrated at the centromeric region of chromosome X, orange fluorescent signals at the centromeric region of the Y chromosome and blue fluorescent signals at the centromeric region of chromosome 18, as well as the blue counterstained chromosomes and nuclei. Enumeration is conducted by microscopic examination of interphase nuclei. The fluorescently-stained regions 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 fluorescent signal corresponds to a copy of the 13q12, 21q22 loci or the centromeric region of chromosomes X, Y or 18.

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® Prenatal Enumeration 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 the 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	14%
Noise-to-signal cut-off percentage	29%
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 13, q12, on chromosome 21, q22.13 and on Xp11.1-q11.1 Mapped on chromosome 18p11.1-q11.1 and on chromosome Yq12.
Practical specificity	100%

Clinical performance

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

Limitations of the Procedure

i) The REMBRANDT® Prenatal Enumeration FISH detection assay is solely applicable for the detection of the 13q12 and 21q22.13 loci and the centromeric region of chromosomes X, 18 and Y, 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, deparaffinization 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 13q12 and 21q22.13 locus and centromeric region of chromosomes X,18 and Y. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® Prenatal Enumeration 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 based on a single test result. A precise diagnosis should consider the clinical history, as well as data obtained by other molecular tests (i.e. WGS).

viii) Therapeutic considerations based on the result of this test alone, should not be 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® 13q12/21q22 and CEPX/CEPY/CEP18	C818P.XXXX	2-8 °C
REMBRANDT® Pepsin powder	R011R.0000	Powder: 2-8°C, ambient temperature Dissolved: -20°C
REMBRANDT® Pepsin diluent	R018R.0000	Concentrated solution and diluted: 2-25°C, ambient temperature
REMBRANDT® PanWash, 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

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