DATA SHEET-V3
REMBRANDT® HER2 AMPLIFICATION FISH DETECTION

CE IVD

PanPath

Ref

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C801K.5206.10	10	T

Intended use

I. The REMBRANDT® HER2 amplification FISH detection assay is an *in-vitro* diagnostics medical device intended for the detection of gene amplification of the locus 17q12 compared to copy numbers of chromosome 17 by means of *in situ* hybridization.

II. The REMBRANDT® HER2 amplification FISH detection assay is intended for the detection of the locus 17q12 compared to copy numbers of chromosome 17 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® HER2 amplification FISH detection assay kit is a quantitative assay for the detection of the locus 17q12 and chromosome 17 copy numbers.

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

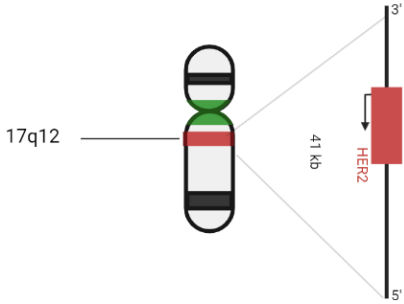
Clinical relevance

The REMBRANDT® HER2 (ERBB2) Human Epidermal growth factor Receptor 2 gene, located at chromosome 17 position q12. HER2 is a member of the Epidermal Growth Factor (EGF) receptor family. HER2 is amplified in 20-30% of breast cancers and has been correlated with a poor prognosis for the patient. Patients carrying the amplification of HER2 can be treated using the monoclonal antibody Herceptin® (Trastuzumab) has been shown to be effective, increasing survival time by specifically targeting cells overexpressing HER2 and removing them from the system. Similar results have been obtained for a variety of other malignant neoplasms overexpressing HER2 including ovarian, stomach, salivary gland and small cell lung cancers (Xu et al., 2019).

Probe specification

The REMBRANDT® HER2 amplification probes mix consists of a dsDNA probe detecting the 17q12 locus and a dsDNA centromeric probe detecting the centromeric region of chromosome 17. The centromeric region is detected by green fluorescence (AF488) and the locus is detected by orange fluorescent detection (AF555).

The REMBRANDT® HER2 amplification probe is able to completely cover the HER2 gene with flanking sequences on the 5' and 3' of the gene for signal enhancement. The REMBRANDT® HER2 amplification 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® HER2 amplification 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® LSI 17q12/CEP17 FISH probe mix	C801P.5206 or C801P.5206.05 or C801P. 5206.10	20 T / 5 T / 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 solution	R026R.0000	15 ml
REMBRANDT® Fluorescent Mounting medium	Z000R.0050	1 ml

Assay procedure

REMBRANDT® HER2 amplification FISH 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 FISH.

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 FISH (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 (C801P.5206.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-FISH). 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. Incubate the slides in PBS at room temperature for 1 minute.

X. 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).

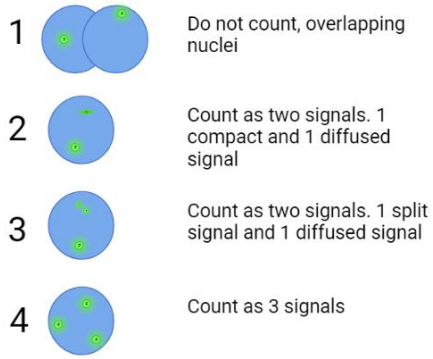
XI. Mount the slides by applying mounting medium (Z000R.0050) and coverslip.

Interpretation of results

Hybridization of the REMBRANDT® HER2 amplification 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 17q12 locus of chromosome 17 in combination with green fluorescent signals representing the centromeric region of chromosome 17. The enumeration HER2 copy numbers of conducted by microscopic examination of interphase nuclei, compared to the green signals representing chromosome 17. The fluorescently-stained locus of chromosome 17 stand out brightly against the general fluorescence of the nucleus. The HER2 amplification procedure enables visual enumeration of copy numbers of the 17q12 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 orange fluorescent signal corresponds to a copy of the 17q12 locus.

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)



Interpretation of HER2 status

The scoring criteria of the REMBRANDT® HER2 amplification FISH detection assay are based on the "Human Epidermal Growth Receptor 2 Testing in Breast Cancer: ASCO/CAP Clinical Practice Guideline Focused Update" (Wolff et al., 2018).

Score according to the following guidelines:

- Score only invasive tumour cells.
- Select a distinct area of invasive tumour and count signals in 20 cells
- Count number of signals per nucleus for both LSI Her2/neu and CEP17.
- Determine ratio of LSI Her2/neu : CEP17 signals per nuclei. The ratio is used to correct for polysomy*.

Assess according to the following guidelines:

- The tumour is amplified if the Her2/neu : CEP17 ratio is 2 or greater, in combination with Her2/neu copy numbers of 4 or greater (group 1).
- The tumour is non-amplified if Her2/neu : CEP17 ratio is less than 2, in combination with less than 4 Her2/neu copy numbers (group 5).
- In all other cases (group 2, 3, 4)**, additional IHC should be performed and scored according to Wolff et al., 2018.

**Rationale: Normal cells in G_0 or G_1 cell cycle phase have 2 copies of each chromosome and therefore will have 2 signals for both chromosome 17 and Her2/neu gene. Replicating cells in G_2 cell cycle phase will have 4 copies and thus 4 signals for both chromosome 17 and Her2/neu gene. Thus, tumour cells can have more than 2 signals without amplification; ratio determination corrects for this.*

Performance characteristics

Analytical Sensitivity and Specificity

The analytical sensitivity and specificity were investigated within PanPaths analytical performance assessment. Precision was investigated for the REMBRANDT® HER2 amplification 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 determined 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	11%
Noise-to-signal cut-off percentage	23%
Hybridization efficiency	99%

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 17q12 and the centromeric region 17p11.1-q11.1
Practical specificity	100%

Limitations of Procedure

i) The REMBRANDT® HER2 amplification FISH detection assay is solely applicable for the detection locus 17q12, 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 17 and the 17q12 loci. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® HER2 amplification 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® LSI17q12/CEP17 FISH probe mix	C801P.5206.XX	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 solution	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 *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

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

Wolff, A. C., Elizabeth Hale Hammond, M., Allison, K. H., Harvey, B. E., Mangu, P. B., Bartlett, J. M. S., Bilous, M., Ellis, I. O., Fitzgibbons, P., Hanna, W., Jenkins, R. B., Press, M. F., Spears, P. A., Vance, G. H., Viale, G., McShane, L. M., & Dowsett, M. (2018). Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/ college of American pathologists clinical practice guideline focused update. *Journal of Clinical Oncology*, *36*(20), 2105–2122. https://doi.org/10.1200/JCO.2018.77.8738

Xu, Z., Xu, P., Fan, W., Huang, B., Cheng, Q., Zhang, Z., Wang, P., & Yu, M. (2019). The effect of an alternative chromosome 17 probe on fluorescence in situ hybridization for the assessment of HER2 amplification in invasive breast cancer. *Experimental and Therapeutic Medicine*, 2095–2103. https://doi.org/10.3892/etm.2019.7756

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