DATA SHEET-V3
REMBRANDT® MDM2 AMPLIFICATION FISH DETECTION

CE IVD

Pan Path

Ref

C817K.2030.05	Σ	5 T
C817K.2030.10	Σ	10 T

Intended use

I. The REMBRANDT® MDM2 amplification FISH detection assay is an *in-vitro* diagnostics medical device intended for the detection of gene amplification of the locus 12q15 compared to copy numbers of chromosome 12 by means of *in situ* hybridization.

II. The REMBRANDT® MDM2 amplification FISH detection assay is intended for the detection of the locus 12q15 compared to copy numbers of chromosome 12 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® MDM2 amplification FISH detection assay kit is a quantitative assay for the detection of the locus 12q15 and chromosome 12 copy numbers.

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

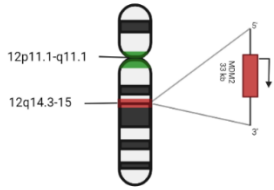
Clinical relevance

Mouse Double Minute type 2 (MDM2) is a known oncogene which is a negative regulator of p53. The MDM2 gene is located on chromosome 12 on position 14.3-15 on the q-arm. Under normal circumstances, MDM2 is involved in two major processes involving p53: it targets p53 for ubiquitin-mediated degradation and it exports p53 from the nucleus to the cytoplasm. Overexpression of MDM2 causes both processes to be overactivated, causing cell proliferation. P53 normally halts cell cycle progression and induces cell death, but overexpression of MDM2 deregulates p53's normal function (Oliner et al., 2016). MDM2 amplifications are associated with many malignancies including glioblastoma, well-differentiated liposarcoma, breast carcinoma, adenocarcinoma (My cancer genome, n.d.).

Probe specification

The REMBRANDT® MDM2 amplification probes mix consists of a 658 kb probe detecting the 12q15 locus and a 0.685 kb centromeric probe detecting the centromeric region of chromosome 12.

The centromeric region is detected by green fluorescence (AF488) and the locus is detected by orange fluorescent detection (AF555). The REMBRANDT® MDM2 amplification probe is able to completely cover the MDM2 gene with flanking sequences on the 5' and 3' of the gene for signal enhancement. The REMBRANDT® MDM2 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® MDM2 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 probe (depending on size choice)		
REMBRANDT® LSI 12q15/CEP12 FISH probe mix	C817P.2030.05 or C817P.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® Pre-treatment buffer	R026R.0000	15 ml
REMBRANDT® Fluorescent Mounting medium	Z000R.0050	1 ml

Assay procedure

REMBRANDT® MDM2 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 (C817P.2030.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 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).

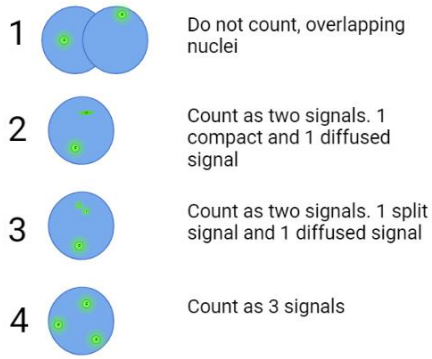
XI. Mount the slides by applying mounting medium (Z000R.0050) and coverslip.

Interpretation of results

Hybridization of the REMBRANDT® MDM2 amplification probe 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 12q15 locus of chromosome 12 in combination with green fluorescent signals representing the centromeric region of chromosome 12. The enumeration MDM2 copy numbers of conducted by microscopic examination of interphase nuclei, compared to the signals representing chromosome 12. The fluorescently-stained locus of chromosome 12 stand out brightly against the general fluorescence of the nucleus. The MDM2 amplification procedure enables visual enumeration of copy numbers of the 12q15 locus compared to chromosome 12 copy numbers within the nuclei. 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)



MDM2 status interpretation

The REMBRANDT® MDM2 amplification FISH detection assay is reported as positive/amplified in case MDM2 clusters are visible at 20/40x magnification. In this case, count at least 20 cell nuclei, if the LSI 12q15/CEP12 ratio is >2, with CEP12 ≥2, the sample contains an amplification of the MDM2 gene.

The REMBRANDT® MDM2 amplification FISH detection assay is reported as negative/not amplified in case the MDM2/nucleus ratio is ≤2. In this case, count at least 20 cell nuclei. If the LSI 12q15/CEP12 ratio is ≤2, the sample does not contain an amplification of the MDM2 gene.

In case of an unclear amplification, or negative sample: no MDM2 clusters, MDM2/nucleus ratio >2, count at least 50 cells, and re-evaluate according to the above described criteria (Gambella et al., 2023).

Performance characteristics

Analytical Sensitivity and Specificity

The analytical sensitivity and specificity were investigated within PanPaths analytical performance assessment. Precision was investigated for the MDM2 amplification 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	10%
Noise-to-signal cut-off percentage	18%
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 the locus 12q15 and the centromeric region 12p11.1-q11.1
Practical specificity	100%

Limitations of Procedure

i) The REMBRANDT® MDM2 amplification FISH detection assay is solely applicable for the detection locus 12q15, 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 locus 12q15 and the centromeric region of chromosome 12. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® MDM2 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® LSI 12q15/CEP12 Probe mix	C817P.2030.XX	2-8 ˚C
REMBRANDT® Pepsin powder	R011R.0000	Powder: 2-25˚C, ambient temperature
REMBRANDT® Pepsin diluent	R018R.0000	Dissolved: -20˚C 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).

Diagnostic Assessment of MDM2 Amplification in Liposarcoma: Potential Pitfalls and Troubleshooting Recommendations. *International Journal of Molecular Sciences*, *24*(2). https://doi.org/10.3390/ijms24021342

My cancer genome. (n.d.). *My cancer genome*. 2020.

Oliner, J. D., Saiki, A. Y., & Caenepeel, S. (2016). The Role of MDM2 Amplification and Overexpression in Tumorigenesis. *Cold Spring Harbor Perspectives in Medicine*, *6*(6), a026336. https://doi.org/10.1101/cshperspect.a026336

Gambella, A., Bertero, L., Rondón-Lagos, M., Verdun Di Cantogno, L., Rangel, N., Pitino, C., Ricci, A. A., Mangherini, L., Castellano, I., & Cassoni, P. (2023). FISH Diagnostic Assessment of MDM2 Amplification in Liposarcoma: Potential Pitfalls and Troubleshooting Recommendations. *International Journal of Molecular Sciences*, *24*(2). https://doi.org/10.3390/ijms24021342

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