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DDIT3 (CHOP) Break Apart FISH Probe

REF **Ref. MAD-017VSBC** **20 tests**

For *in vitro* diagnostic use
Regulation (EU) IVDR 2017/746

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1 INTENDED PURPOSE OF THE PRODUCT

The DDIT3 (CHOP) Break Apart FISH Probe is a qualitative, automated *in vitro* diagnostic test intended to be used as an aid in the diagnosis of diseases related to DDIT3 gene rearrangements in formalin-fixed, paraffin-embedded (FFPE) human tissue specimens using fluorescence *in situ* hybridization (FISH). DDIT3 gene rearrangements are associated with myxoid liposarcoma.

This test should be performed under the supervision of healthcare professionals trained in molecular and cytogenetic test interpretation. It is not intended to be used as a sole diagnostic procedure but should be interpreted in conjunction with other clinical and laboratory information.

2 SUMMARY AND INTRODUCTION

Myxoid and round cell liposarcomas (MRCLs) are the most common subtypes of liposarcoma. Compared to other forms of liposarcoma, MRCLs have a greater tendency to metastasize. Rearrangements and abnormal expression of the DDIT3 gene also known as CHOP, CEBPZ, CHOP10, CHOP-10, or GADD153 have been observed in myxoid liposarcomas and other malignancies. One of the most common genetic abnormalities is the reciprocal translocation t(12;16)(q13;p11), which is present in up to 95% of cases. This translocation results in the FUS-DDIT3 fusion protein, which has oncogenic potential and interferes with adipocytic differentiation. EWSR1 is an alternative translocation partner of the DDIT3 gene and the resulting fusion protein EWSR1-DDIT3, which originates in t(12;22)(q13;q12), is present in <5% of cases of MRCLs. The DDIT3 rearrangements are highly specific for MRCLs due to the presence of the FUS-DDIT3 or EWSR1-DDIT3 fusion genes.

2.1 Probe Design

The DDIT3 Break Apart FISH probe is designed to detect rearrangements involving the human DDIT3 gene, located at chromosomal band 12q13.3. The DDIT3 Break Apart FISH probe consists of two fluorescently labeled DNA fragments. The orange probe hybridizes to sequences upstream (5′) of the DDIT3 gene, covering adjacent flanking genomic regions. The green probe hybridizes to sequences downstream (3′) of the DDIT3 gene, encompassing nearby flanking regions. The DDIT3 gene itself lies between these two probes. Both probes cover regions where recurrent chromosomal breakpoints have been identified, enabling reliable detection of structural alterations involving this locus.

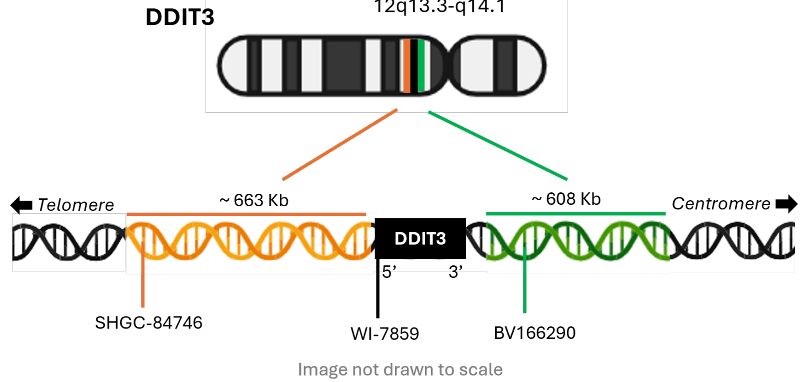

Figure 1. Schematic representation of the DDIT3 (CHOP) Break Apart FISH Probe targeting the 12q13.3-q14.1 region.

2.2 Excitation and Emission Parameters

DNA PROBE	REGION	FLUOROCHROME	EXCITATION	EMISSION
3´DDIT3 GREEN PROBE	Downstream of DDIT3 gene	Green	491 nm	516 nm
5´DDIT3 ORANGE PROBE	Upstream of DDIT3 gene	Orange	552 nm	576 nm

Vitro FISH probes are optimized for use with standard fluorescence microscopy filter sets matched to these wavelengths. Using filters outside this range may result in suboptimal visualization.

3 PRINCIPLE OF THE METHOD

The DDIT3 (CHOP) Break Apart FISH Probe is intended for use in automated fluorescence *in situ* hybridization (FISH) procedures on formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The methodology involves the following key steps:

1. **Pretreatment and Hybridization**: FFPE tissue sections undergo deparaffinization, rehydration and enzymatic digestion, followed by DNA denaturation to facilitate probe binding. The ready-to-use DDIT3 (CHOP) Break Apart FISH Probe, labeled with fluorophore molecules, hybridizes specifically to the target DNA sequence within the sample. Hybridization is carried out under controlled temperature conditions.
2. **Washing and Counterstaining**: Following hybridization, a series of wash steps are employed to remove non-specifically bound probes, ensuring the specificity of the signal. The sample is then counterstained with DAPI, a fluorescent dye that binds to DNA in the cell nuclei, allowing for the differentiation of FISH signals in the context of cellular structures.
3. **Microscopy and Analysis**: Fluorescent signals from the hybridized probes are visualized and analyzed using fluorescence microscopy with the appropriate filters. The emitted fluorescence enables localization of the target DNA sequences, facilitating the detection of genetic alterations such as amplifications or deletions. Analysis can be performed manually or with image analysis software designed for quantitative and qualitative signal assessment.

4 RECONSTITUTION, MIXING OR DILUTION

This product is provided in a ready-to-use format. It is not necessary to reconstitute or dilute it.

5 COMPONENTS

The **DDIT3 (CHOP) Break Apart FISH Probe** kit is commercialized as a ready-to-use product. This probe is optimised for use in NeoPATH Pro.

NAME	VITRO, S.A. Ref.	BIOCARE Ref.	CONTENT	QUANTITY	Nº TEST
DDIT3 (CHOP) Break Apart FISH Probe	MAD-017VSBC	EG007-VTR-FP-CE-NP	FISH Probe	1 vial (2.51 mL)	20

Table 1. Reagents supplied in the product.

6 ADDITIONAL REQUIRED MATERIAL NOT SUPPLIED

A list of the materials, reagents and equipment necessary for the use of DDIT3 (CHOP) Break Apart FISH Probe that are not included in it is shown below.

6.1 Reagents and materials

- Cover, Biocare Ref NPRI10002L2, Vitro Ref. MAD-003983VS
- DEWAX-2 FISH, Biocare Ref NPRI10050T200, Vitro Ref. MAD-004081VS
- FISH PT KIT, Biocare Ref NPRI10058KT40, Vitro Ref. MAD-004082VS
- High AR, Biocare Ref NPRI10003L2, Vitro Ref. MAD-004075VS
- TBS Tween 20 Buffer 10X, Biocare Ref NPRI10007MM, Vitro Ref. MAD-004077R-10
- Cleaning Solution 10X, Biocare Ref NPRI10008MM, Vitro Ref. MAD-003931CSVS
- Probe Cleaning Kit, Biocare Ref NPRI10009KC10, Vitro Ref. MAD-PCLK
- IHC Treated Slides, Biocare Ref NPRI10011, Vitro Ref. MAD-15-188-55/100
- DAPI

6.2 Equipment

- NeoPATH Pro
- Fluorescence microscope

7 STORAGE AND STABILITY CONDITIONS

Component	Use conditions
Storage conditions	Store at 2°-8°C.
In-use stability	Once opened, store at 2-8°C until the expiration date of the product.
Shipping conditions	Shipment should be performed at 2-8°C.

Table 2. Storage and stability conditions.

The product is stable to the expiration date printed on the label when stored at 2°-8°C. Do not use after expiration date.

8 WARNINGS AND PRECAUTIONS

- **Read the instructions for use before using this product**. In case of atypical or unexpected results, please contact your Authorized Supplier/Distributor.
- **Professional Use**. This product is only intended for professional laboratory purposes, and it is not intended for pharmacological, domestic or any other type of use. When the product is used as an aid to diagnosis it should only be handled by trained users and in authorized laboratories and strictly following the instructions contained in this brochure.

- **Use**:
  - Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.
  - Microbial contamination of reagents may result in an increase in nonspecific staining or/and erroneous results.
  - Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
  - Do not use reagent after the expiration date printed on the label.
- **Serious incident.** Any serious incident related to the use of this product that involves or may involve a serious deterioration, temporary or permanent, of the state of health of a patient, user or other person, or even death, or a serious threat to public health, must be reported as soon as possible to the manufacturer by e-mail at regulatory@vitro.bio and to the competent Health Authority of the EU member state where the user or patient is established. Incidents caused by misuse of the product or by the use of the product beyond the useful life established on its labeling will be the responsibility of the user.
- **The safety and disposal precautions are described in the Safety Data Sheet of this product.** This product is only intended for professional laboratory purposes, and it is not intended for pharmacological, home or any other type of use. The current version of the Safety Data Sheet of this product can be downloaded in the web page www.vitro.bio or requested at regulatory@vitro.bio.
- **Summary of Safety and Performance (SSP):** The Summary of Safety and Performance (SSP) of this product will be available on EUDAMED database (subject to EUDAMED availability). This summary is also available on request by e-mail at regulatory@vitro.bio.
- **Recommendations for the Safe Handling of Samples Suspected of Containing Prions:**

  The following protective measures are recommended for professional personnel handling *in situ* hybridization (ISH) samples suspected of containing prions:
  - Personal protective equipment (PPE): full PPE should be worn, including a mask (preferably FFP2 or higher), protective goggles or face shield, head cover, and shoe covers.
  - Precautions during microtome sectioning: use disposable blades and avoid the use of aerosols or compressed air to clean the microtome area.
  - Surface and equipment decontamination: disinfect with sodium hydroxide (NaOH) 1N or sodium hypochlorite at 2%.
  - Waste management: all disposable materials must be discarded as special biological risk waste, in accordance with the applicable national regulations on hazardous healthcare waste.
- **Waste disposal:** The handling of waste generated by the use of the products commercialized by VITRO S.A. must be performed according to the applicable law in the country in which these products are being used. As reference, the following table indicates the classification of waste generated by this kit according to the European Law, specifically according to the *European Commission Decision of December 18, 2014,* amending decision 2000/532/CE on the list of waste pursuant to Directive 2008/98/EC of the European Parliament

and of the Council:

POTENTIAL WASTE GENERATED AFTER USING THIS PRODUCT	ELW* CODE	TYPE OF WASTE ACCORDING TO ELW*
Container for reagents used classified as dangerous (according to the Safety Data Sheet).	150110	"Containers containing waste or contaminated by dangerous substances"
Aqueous liquid waste containing hazardous substances (not solvents).	161001	"Liquids generated from the use of automatic IHC/HIS instruments: - Waste deposit of immunostainers. - used PT-Module buffers"
Consumables (tubes, tips, etc.). Any element that has been in contact with tissue samples.	180103	"Waste whose collection and disposal is subject to special requirements in order to prevent infection"
Liquids containing solvents (xylol, haematoxylin, alcohol, eosin), generated from immunostaining techniques.	160506	"Laboratory chemicals consisting of or containing dangerous substances, including mixtures of laboratory chemicals"

Table 3. Classification of waste generated by this kit according to the European Legislation. *ELW: European Legislation of Waste.

***Note: This classification is included as a general guideline of action, being under the final responsibility of the user the accomplishment of all the local, regional and national regulations on the disposal of this type of material.**

9 PREPARATION OF THE SAMPLE FOR ANALYSIS

Formalin-fixed, paraffin-embedded (FFPE) tissue should be cut into 4–6 µm sections and mounted on treated (positively charged) microscope slides. Bake the slides at 60 °C for about 60 minutes to soften the paraffin and help the sections adhere firmly to the slide.

The prepared slides can be stored at 2–8 °C for up to 12 months before running the test. Keep them protected from light and moisture.

Once the FISH procedure is complete (including the post-hybridization washes and counterstaining), store the slides at –20 °C, protected from light, to preserve the fluorescent signal and reduce fading. Before analysis, let the slides warm to room temperature.

10 PROTOCOL

The FISH probe is intended for automated *in situ* hybridization on formalin-fixed, paraffin-embedded (FFPE) tissue sections using the NeoPATH Pro system. Select the appropriate protocol from the instrument's menu and follow the on-screen instructions to ensure proper sample treatment and hybridization.

⚠ **Prior to instrument loading, ensure the probe is homogeneously mixed by gently pipetting the reagent up and down 10 – 20 times. Insufficient mixing may negatively affect assay performance and signal quality.**

11 INTERPRETATION OF RESULTS

Before interpreting the results of the clinical samples, it is necessary to follow the interpretation guide of the positive and negative samples as in the images below:

Normal signal pattern:

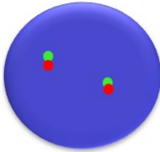

Figure 2. Expected signals pattern observed in a normal cell.

Abnormal signal pattern:

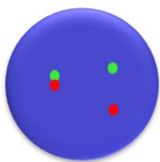

Figure 3. Expected signal pattern observed in a cell with DDIT3 (CHOP) rearrangement.

Additional signal patterns may be observed in aneuploid or unbalanced specimens due to variations in chromosome number or structural abnormalities.

For the interpretation of fluorescence hybridisation results it is recommended to apply the current internationally accepted interpretation criteria.
Prior to analysis, the quality of the sample should be assessed according to the following criteria:

- Fluorescent signals should be bright, distinct, and easily evaluable.
- Greater that 50% of the cells should show hybridisation signals.
- Background should appear dark/black and clean.
- Nuclear borders should be clearly defined and intact.
- Clustered or overlapping cells should be minimized, as they may interfere with accurate analysis.

The following guidelines should be considered for signal enumeration and results interpretation:

- Only morphologically preserved nuclei should be analysed. Nuclei that are overlapped or covered by cytoplasmic debris or exhibit a high degree of autofluorescence should be excluded.
- Areas with excessive cytoplasmic debris or non-specific hybridisation should be avoided.
- If two signals of the same colour are in contact, separated by a distance less than or equal to two signal diameters, or connected by a faint strand, they should be count as one signal.

- When orange and green signals are separated by a distance of no more than two signal diameters, they should be considered a fused (non-rearranged) signal.
- If there is uncertainty regarding the analyzability of a cell, it should be excluded from the evaluation to maintain the integrity of the results.

12 ANALYTICAL PERFORMANCE OF THE PRODUCT

12.1 Analytical sensitivity

The probe was tested on FFPE sections from 10 karyotypically normal individuals (400 nuclei per individual). An analytical sensitivity of 97.20% was achieved, meeting the acceptance criterion of ≥95%.

12.2 Analytical specificity

Analytical specificity was assessed using metaphase spreads from the MRC-5 cell line. All 50 target loci showed correct signal localization, yielding a specificity of 100%, meeting the predefined criterion of ≥98%.

12.3 Repeatability

Repeatability was evaluated using six replicates of FFPE sections from a normal specimen under identical conditions. The coefficient of variation (CV) was 1.38%, meeting the acceptance criterion (CV≤10%).

12.4 Reproducibility

Reproducibility was assessed across two different conditions (inter-lab variability) with three replicates each. The resulting CV was 1.38%, also within the acceptance criterion (CV≤10%), confirming consistent performance across test conditions.

12.5 Cut-off value

The cut-off was determined from over 4000 nuclei of normal samples using statistical analysis (BETAINV function). The established cut-off value is 3.40%.

13 CLINICAL PERFORMANCE OF THE PRODUCT

The clinical performance of the DDIT3 (CHOP) Break Apart FISH Probe was evaluated in a retrospective study using 30 FFPE tissue samples from various anatomical sites (soft tissues, mesentery, mediastinum and pericardium). All samples had been previously tested with a CE-IVD marked reference methods (Cytotest™ DDIT3 Break Apart FISH Probe Kit or Abbot Vysis DDIT3 Break Apart FISH Probe Kit).

The study included 20 DDIT3-rearrangement-positive and 10 negative samples, confirmed by the reference method. Diagnoses among positive cases included myxoid liposarcoma, spindle cell liposarcoma, dedifferentiated liposarcoma, liposarcoma and fibromyxoid mesenchymal lesion.

Clinical Performance Results:

- Diagnostic Sensitivity: 100% (95% CI: 83.16–100%)
- Diagnostic Specificity: 100% (95% CI: 69.15–100%)
- Positive Predictive Value (PPV): 100% (95% CI: 83.16–100%)
- Negative Predictive Value (NPV): 100% (95% CI: 69.15–100%)
- Agreement: 100%
- Kappa Coefficient: 1.0 (95% CI: 100–100%), indicating *very good* agreement

The DDIT3 (CHOP) Break Apart FISH Probe demonstrated 100% sensitivity, specificity, PPV, and NPV in this clinical study, meeting all predefined acceptance criteria. The probe provides reliable detection of DDIT3 (CHOP) gene rearrangements in FFPE samples, supporting its clinical utility in diagnosis and prognosis for patients with myxoid liposarcoma.

14 PRODUCT LIMITATIONS

- Results must be interpreted by qualified healthcare professional in the context of patient´s clinical history, symptoms, and other diagnostic findings.
- The correct performance of the test depends on the quality of the sample.
- FISH is a multistep, technically complex procedure requiring specialized training in tissue selection, fixation, processing, reagent selection, preparation of the FISH slide and interpretation of fluorescent signals.
- Tissue preparation is critical. Improper fixation, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or tissue heterogeneity.
- FISH results must be interpreted in conjunction with morphological evaluation and other diagnostic tests. The kit is intended as an adjunctive tool and not as a stand-alone diagnostic or screening test. Therapeutic decisions should not be based solely on FISH results.
- Each laboratory should test sufficiently large number of samples to establish normal population distribution of the signal levels and to assign a cut-off value.
- The clinical interpretation of any positive or negative result should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive or negative result should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.
- This reagent must be used according to the manufacturer's protocol for FISH on formalin-fixed, paraffin-embedded (FFPE) tissue sections. Deviation from the recommended procedure may invalidate the results. Laboratories deviating from the protocol assume responsibility for interpreting results under those conditions.
- The probe detects genomic alterations only within its specific target region. Rearrangements outside this region may not be detected.

15 TROUBLESHOOTING

Problem	Possible causes	Recommended Solutions
No hybridization or weak signals	Failure in the FISH protocol.	Verify that the NeoPATH Pro is functioning correctly and that all reagents were added properly. Repeat the test.
	FISH reagents expired or improperly stored.	Check expiration dates and storage conditions of all reagents. Replace as necessary and repeat the test.

16 BIBLIOGRAPHY

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17 LABEL AND BOX SYMBOLS

Symbol	Meaning	Symbol	Meaning
IVD	*In vitro* diagnostic medical device		Expiration date
REF	Catalog number		Temperature limit
LOT	Lot code		Manufacturer
	Refer to the instructions for use	Σ	Sufficient content for <n> assays
e-SDS	Safety data sheet		Distributor
	Importer		Keep away from sunlight

18 CHANGELOG

Date	Description
04/03/2026	Initial elaboration of the document.