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DEWAX-2 FISH

For *In vitro* Diagnostic Use

1 AVAILABLE PRODUCT FORMATS

Name	VITRO, S.A. Reference	BIOCARE Reference	No. test	Volume
DEWAX-2 FISH	MAD-004081VSBC	NPRI10050T200	200	2L

Table 1. Reference and presentation.

2 INTENDED PURPOSE OF THE PRODUCT

The DEWAX-2 FISH is intended for the removal of paraffin from formalin-fixed paraffin-embedded (FFPE) tissue sections as part of automated fluorescence *in situ* hybridization (FISH) protocols for *in vitro* diagnostic use.

3 SUMMARY AND EXPLANATION

DEWAX-2 FISH is a reagent specifically formulated for the efficient removal of paraffin from formalin-fixed, paraffin-embedded (FFPE) tissue sections. It is intended for use as part of an automated FISH (fluorescence *in situ* hybridization) protocol performed on the NeoPATH Pro automated staining system. Effective deparaffinization is a critical pretreatment step to ensure optimal accessibility of nucleic acid targets during probe hybridization.

4 PRINCIPLE OF PROCEDURE

The **DEWAX-2 FISH** is designed for use in automated FISH workflows on FFPE tissue specimens. The methodology consists of the following main steps:

- **Pretreatment and Hybridization**: FFPE tissue sections undergo deparaffinization, rehydration and enzymatic digestion, followed by DNA denaturation to facilitate probe binding. The ready-to-use FISH Probe, labeled with fluorophore molecules, hybridizes specifically to the target DNA sequence within the sample. Hybridization is carried out under controlled temperature conditions.
- **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.
- **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.

5 RECONSTITUTION, MIXING, DILUTION

DEWAX-2 FISH is a ready-to-use format. It is not necessary to reconstitute, mix or dilute it.

6 ADDITIONAL REQUIRED MATERIAL NOT SUPPLIED

6.1 Reagents and materials

- FISH Probe
- Cover, BIOCARE Ref. NPRI10002L2, Vitro Ref. MAD-003983VS.
- FISH PT KIT, BIOCARE Ref. NPRI10058KT40, Vitro Ref. MAD-004982VS
- 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
- Fluorescent microscope.

7 STORAGE AND STABILITY CONDITIONS

Component:	Use conditions
Storage conditions	Store at 15-25°C until the expiration date.
In-use stability	Once placed on the instrument, the reagent remains stable until the expiration date indicated on the label at 15–25 °C (room temperature).
Shipping conditions	Shipment should be performed at 15-25°C.

Table 2. Storage and stability conditions.

Do not use after expiration date.

8 SPECIMEN PREPARATION

Formalin-fixed paraffin-embedded (FFPE) tissue should be sectioned between 4–6 µm and adhered to treated slides. The slides with the tissue sections have to be placed in an oven at 60 °C to melt the paraffin and allow the tissue to adhere perfectly to the slide.
Tissue sections mounted on charged slides can be held for up to 12 months at 2–8 °C before staining. Following sectioning, it is recommended that slides are incubated at 60 °C for one hour. Stained sections should be stored at -20 °C to preserve fluorescent signal and prevent fading. Allow stored slides to reach room temperature prior to reading.

9 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.
- Rx Only **Physician prescribed test.** This product is for professional use only on prescription by a physician or other healthcare professional.
- **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. If the user is located in USA, report any serious incidents related to this device by contacting the local distributor (information identified on the product labelling) and the applicable competent authority of the Member State. 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.** 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.
- **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:

POTENTIAL WASTE GENERATED AFTER USING THIS PRODUCT	ELW* CODE	TYPE OF WASTE ACCORDING TO ELW*
		- 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.**

10 INSTRUCTIONS FOR USE

Place the kit in the VitroSatiner 42 before starting the FISH process in the instrument. Select the appropriate protocol from the instrument's menu and follow the on-screen instructions to ensure proper sample treatment and hybridization.

11 TROUBLESHOOTING

Follow the NeoPATH Pro instructions for FISH. If atypical results occur, contact Vitro's Regulatory Department at regulatory@vitro.bio. If the user is located in USA, contact the local distributor (information identified on the product labelling).

12 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.

13 PERFORMANCE CHARASTERISTICS

Intra-run reproducibility of Dewax-2 was demonstrated by staining slides containing identical tissue sections within the same run, yielding consistent results across all slides. Inter-run reproducibility was confirmed by staining identical tissue on different days, using different instruments and operators. Consistent performance was observed under all conditions. Additional studies evaluated a wide range of FISH Probes, tissue types, and operational variables over time. Dewax-2 showed reliable and consistent performance across all tested conditions.

14 BIBLIOGRAPHY

- Analysis of genes and chromosomes by nonisotopic in situ hybridization. Lichter P, et al. Genet Anal Tech Appl. 1991 Feb;8(1):24- 35. 2.
- Fluorescence in situ Hybridization (FISH). Bayani J, Squire JA. Curr Protoc Cell Biol. 2004. 3. Clinical and Laboratory Standards Institute (CLSI).
- Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition (M29-A4) Wayne, PA 2014.

15 LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

IVD	*In vitro* diagnostic medical device		Expiration date
REF	Catalog number		Temperature limit
LOT	Lot code		Manufacturer
	Refer to the instructions for use	e-SDS	Safety data sheet
	Distributor		Importer

16 CHANGELOG

Date	Description
2025-08-19	Initial creation of the IFU
2025-08-22	Section “7. Storage and stability conditions” has been updated.