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Low-AR

For *In vitro* Diagnostic Use.

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1 AVAILABLE PRODUCT FORMATS

VITRO, S.A. Ref.	BIOCARE Ref	Presentation
MAD-004079VSBC	NPRI10004L2T50	1 x 35 mL

Table 1. References and presentation

2 INTENDED USE

For in vitro diagnostic use. The ready-to-use Low-AR solution is used in heat-induced antigen retrieval of formalin-fixed paraffin embedded (FFPE) tissue sections as part of the performance of automated immunohistochemistry (IHC) protocols. It is designed for use by qualified professionals trained in immunohistochemistry techniques. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.
This product is intended for people of all ages who require analysis of the detection of antigens by immunohistochemical techniques.

3 SUMMARY AND EXPLANATION

The process of fixation of formalin-fixed, paraffin-embedded tissue section samples can lead to protein cross-linking, which masks antigens and limits antigen-antibody binding. Antigen retrieval allows an antibody to access the target antigen within the tissue since the masked epitopes can be retrieved using heat-induced antigen retrieval (HIER) methods. These methods have greatly improved immunohistochemical antigen staining in conventional surgical pathology. The use of these techniques is most advantageous for nuclear antigens, where a marked increase in immunohistochemical signal intensity has been observed. For some antibodies, the use of buffered solutions for HIER has been found to improve the immunostaining intensity.

The ready-to-use Low-AR solution is used for heat-induced antigen retrieval as part of an IHC staining procedure in the NeoPATH Pro automated stainer.

4 PRINCIPLE OF PROCEDURE

In general, immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (link antibody), an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

5 RECONSTITUTION, MIXING, DILUTION

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

6 SUPPLIED AS

Buffered solution containing non-ionic surfactants, stabilizers and preservatives.

7 ADDITIONAL REQUIRED MATERIAL NOT SUPPLIED

7.1 Reagents and materials

- Primary Antibody
- Cover, BIOCARE Ref. NPRI10002L2, Vitro Ref. MAD-003983VS
- Dewax Solution, BIOCARE Ref. NPRI10001T280, Vitro Ref. MAD-004080VS
- DAB Enhancer, BIOCARE Ref. NPRI10005L2, Vitro Ref. MAD-001560QV
- Contrast Hematoxylin HDH3, BIOCARE Ref. NPRI10006L2, Vitro Ref. MAD-HDH3-100VS
- TBS Tween 20 Buffer 10X, BIOCARE Ref. NPRI10007MM, Vitro Ref. MAD-004077R-10
- Cleaning Solution 10X, BIOCARE Ref. NPRI10008, Vitro Ref. MAD-003931CSVS
- Probe Cleaning Kit, BIOCARE Ref. NPRI10009KC10, Vitro Ref. MAD-PCLK
- Master Polymer Plus Detection System, BIOCARE Ref. NPRI10010T135, Vitro Ref. MAD-000237QKVS
- IHC Treated Slides, BIOCARE Ref. NPRI10011, Vitro Ref. MAD-15-188-55/100
- Mixing Tubes, BIOCARE Ref. NPP20001, Vitro Ref. MAD-60732-100

7.2 Equipment

- NeoPATH Pro
- Optical microscope and/or digital scanner of histological slides

8 STORAGE AND STABILITY CONDITIONS

Component:	Use conditions
Storage conditions	Store at room temperature (15°-25°C) and keep away from sources of intense heat/cold until the expiration date of the product.
In-use stability	Once open, keep at room temperature (15°-25°C) and keep away from sources of intense heat/cold until the expiration date of the product.
Shipping conditions	Shipment should be performed at room temperature (15-25°C).

The product is stable to the expiration date printed on the label when stored at 15°-25°C. Do not use after expiration date.

9 SPECIMEN PREPARATION

Paraffin Sections: Tissues fixed in *formalin* are suitable for use prior to paraffin embedding. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades.

10 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.
- Rx Only **Physician prescribed test.** This product is for professional use only on prescription by a physician or other healthcare professional.
- 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. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
- 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 (SDS) of this product can be downloaded in the web page www.vitro.bio or requested at regulatory@vitro.bio.
- **Waste disposal:** The handling of wastes 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 wastes 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 the use of 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 materials**.

11 INSTRUCTIONS FOR USE

Place the product components according to the NeoPATH Pro software.

12 QUALITY CONTROL

Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures. Consult the quality control guidelines of the Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011.

Positive Tissue Control: External Positive control materials should be fresh autopsy/biopsy/surgical specimens fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared tissues and proper staining techniques. One positive external tissue control for each set of test conditions should be included in each staining run.

The tissues used for the external positive control materials should be selected from patient specimens with well-characterized low levels of the positive target activity that gives weak positive staining. The low level of positivity for external positive controls is designed so to ensure detection of subtle changes in the primary antibody sensitivity from instability or problems with the IHC methodology. Commercially available tissue slides or specimens processed differently from the patient sample(s) validate reagent performance only and do not verify tissue preparation.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control: Use a negative tissue control fixed, processed and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the IHC primary antibody for demonstration of the target antigen, and to provide an indication of specific background staining (false positive staining). Also, the variety of different cell types present in most tissue sections can be used by the laboratorian as internal negative control sites to verify the IHC's performance specifications.

If specific staining (false positive staining) occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Nonspecific Negative Reagent Control: Use a nonspecific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. Ideally, a negative reagent control contains an antibody produced from tissue culture supernatant in the same way as the primary antibody but exhibits no specific reactivity with human tissues in the same matrix/solution as the primary antibody. Diluent alone may be used as a less desirable alternative to the previously described negative reagent controls. The incubation period for the negative reagent control should correspond to that of the primary antibody.

When panels of several antibodies are used on serial sections, the negatively staining areas of one slide may serve as a negative/nonspecific binding background control for other antibodies.

To differentiate endogenous enzyme activity or nonspecific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate-chromogen or enzyme complexes (PAP, avidin-biotin, streptavidin) and substrate-chromogen, respectively.

13 ASSAY VERIFICATION

Prior to initial use of an antibody or staining system in a diagnostic procedure, the user should verify the antibody's specificity by testing it on a series of in-house tissues with known immunohistochemical performance characteristics representing known positive and negative

tissues. Refer to the quality control procedures previously outlined in this section of the product insert and to the quality control recommendations of the CAP Certification Program for Immunohistochemistry and/or the NCCLS IHC guideline). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters.

14 TROUBLESHOOTING

Follow the antibody specific protocol recommendations according to data sheet provided. 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).

15 INTERPRETATION OF RESULTS

Interpretation of the results should be done by a qualified pathologist. Staining with the primary antibody along with ancillary reagents should be performed on patient tissues and on positive and negative controls.

Positive Tissue Control: The positive tissue control stained with the primary antibody should be examined first to ascertain that all reagents are functioning properly. The presence of a reddish-brown (3,3' diaminobenzidine tetrachloride, DAB) reaction product with the target cell's (location in the cell) is indicative of positive reactivity. If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid.

Negative Tissue Control: The negative tissue control should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross-reactivity to cells/cellular components**.** If specific staining (false positive staining) occurs in the negative external tissue control, results with the patient specimen should be considered invalid.

Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Patient Tissue: Examine patient specimens stained with the primary antibody last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

Refer to the instructions for use of the primary antibody for information about the immunoreactivity of the antibody.

16 LIMITATIONS

- The results of the test must be evaluated by a healthcare professional in the context of medical history, clinical symptoms, and other diagnostic tests.
- The correct performance of the test depends on the quality of the sample.
- NeoPATH Pro and the ancillary reagents should be used with formalin-fixed paraffin-embedded tissue sections. The use of any other type of sample may generate erroneous results and its performance must be verified beforehand.
- Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
- Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.
- Excessive or incomplete counterstaining may compromise proper interpretation of results.
- The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents and methods to interpret all of the steps used to prepare and interpret the final IHC preparation.
- The manufacturer provides this reagent for use following the provided instructions for IHC on prepared tissue sections. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.
- Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.

17 PERFORMANCE CHARASTERISTICS

The intra-run and inter-run reproducibility of the Low-AR reagent performance was evaluated by staining slides containing the same tissue on two different instruments, with two operators, and on different days. The staining results were consistent across all conditions, demonstrating 100% concordance in tissue samples and performing as expected.

18 BIBLIOGRAPHY

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19 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		Safety data sheet
	Distributor		Importer

20 CHANGELOG

Date	Description
2025-02-27	Creation of the document.
2025-03-31	The volume and the Biocare reference in Table 1 has been updated.
2025-04-09	Section 11 “Instructions for use” has been updated.
2025-04-10	The volume data in table 1 has been corrected.