VITRO S.A
Calle Luis Fuentes Bejarano 60
Edificio Nudo Norte Local 3
41020 Seville (Spain)
F: +34 954 922 892
P: +34 954 933 200
www.vitro.bio

Master Polymer Plus Detection System (Peroxidase)

Table of contents

1 CATALOG REFERENCES AND PRESENTATIONS

The references and presentations for these products (supplied as kits), as well as the quantity of components supplied for each of them, are shown in the following table:

	Reference	MAD-000237QP-100/V	MAD-000237QP-400/V
	No. of Tests	100	400
Components of the Kit		**Quantity**	**Quantity**
Peroxidase blocking reagent		1 x 100 test	4 x 100 test
Primary Antibodies Amplifier Master		1 x 100 test	4 x 100 test
Master Polymer Plus HRP		1 x 100 test	4 x 100 test
DAB Chromogen 1x		1 x 115 test	4 x 115 test
DAB Substrate 1x		1 x 115 test	4 x 115 test
DAB Enhancer		1 x 100 test	4 x 100 test
Contrast Hematoxylin HDH3		1 x 100 test	4 x 100 test

2 INTENDED PURPOSE OF THE PRODUCT

The product Master Polymer Plus Detection System (Peroxidase) is an immunohistochemistry developer kit. It is an *in vitro* diagnostic product for professional use used for the detection of monoclonal and polyclonal primary antibodies obtained in mice and monoclonal and polyclonal primary antibodies obtained in rabbits. The kit is designed for laboratory use by automated immunohistochemistry on MD-Stainer instruments on paraffin-embedded tissue in neoplasms and in all other clinicopathological and physiological situations where the identification of antigens by immunohistochemical techniques is necessary.

The Master Polymer Plus Detection System (Peroxidase) is intended for use in the pathology laboratory for the *in vitro* detection by immunohistochemical procedures of antibodies to specific antigens in human tissues (use in other species has not been evaluated) fixed in buffered formalin and embedded in paraffin-embedded formalin (FFPE).

3 SUMMARY AND EXPLANATION

Manual quantification of the immunostaining obtained by percentage count of positive cells or per square mm on a grid is highly recommended as an alternative to semiquantitative assessment and, even better, its eventual quantitative assessment by automated morphometry and/or computational pathology systems if, after validation, they have been enabled in the client laboratory.

Regarding the measurement range of the antibody immunostaining to be developed, which is valid for any of the immunohistochemical procedures eventually employed in its detection, it is proposed that the immunostaining obtained be assessed by one of the semiquantitative procedures already designed previously in the literature to be applied to immunohistochemical studies. These procedures must be implemented by each client laboratory. Among them, those that adopt valuation systems based on at least five degrees of intensity are more recommendable.

Positive and negative results obtained using antibodies developed with the Master Polymer Plus Detection System (Peroxidase) can aid in the identification and classification of normal and pathological cells and tissues and serve to complement the data provided by conventional histopathology. The clinical interpretation of any positive staining or lack thereof should be complemented by as many morphological and histological studies with appropriate controls as necessary or as explicitly indicated for this type of study.

The evaluation of immunostaining should be performed by a qualified pathologist within the context of the clinical history and the remaining analytical, imaging or immunohistochemical diagnostic tests performed on the patient. In addition, this product is not used as an individual diagnostic piece or kit since, on the one hand, its result is dependent on other reagents and processes that together constitute the Immunohistochemistry technique and, on the other hand, its use is highly recommended as part of a broader panel of antibodies to support the diagnosis.

4 DESCRIPTION OF THE MOLECULE IDENTIFIED BY THE PRODUCT

The product recognizes the isotype of the immunoglobulins corresponding to the primary antibody. The analyte to be determined will depend on the primary antibody used. See Instructions for Use for the specific primary antibody to be developed with the kit Master Polymer Plus Detection System (Peroxidase).

5 EXPRESSION IN NORMAL TISSUES

Expression in normal tissues will be determined by the primary antibody used. See Instructions for Use for the specific primary antibody to be developed with the kit Master Polymer Plus Detection System (Peroxidase).

6 DIAGNOSTIC, PROGNOSTIC AND PREDICTIVE AID APPLICATIONS

6.1 Diagnostic aid

The diagnostic aid will be determined by the primary antibody used. See Instructions for Use for the specific primary antibody to be developed with the kit Master Polymer Plus Detection System (Peroxidase).

6.2 Possible indications of prognostic and predictive use

The eventual indications for prognostic and predictive use will be determined by the primary antibody used. See Instructions for Use for the specific primary antibody to be developed with the kit Master Polymer Plus Detection System (Peroxidase).

6.3 Clinical specificity and sensitivity

Clinical specificity and sensitivity will be determined by the primary antibody used. See Instructions for Use for the specific primary antibody to be developed with the kit Master Polymer Plus Detection System (Peroxidase).

7 DEVICE DESCRIPTION

7.1 Composition

The Master Polymer Plus Detection System (Peroxidase) is a kit whose components are:

Description	Reference
Peroxidase Blocking Reagent	MAD-021540Q-100
Primary Antibodies Amplifier Master	MAD-000237QP –A/100
Master Polymer Plus HRP	MAD-000237QP –B/100
DAB Substrate 1X	MAD-001813QS-100
DAB Chromogen 1X	MAD-001813QC-100
DAB Enhancer	MAD-001560Q-100
Contrast Hematoxylin HDH3	MAD-HDH3-100

All the components are provided in liquid and in a ready-to-use format.

Notes: The DAB mix (DAB Chromogen 1X +DAB Substrate 1X) is prepared in the rack of reagents right before using it.

When executing a staining cycle of less than 8 preparations, the system will prepare a minimum volume corresponding to 8 tests to guarantee the volume necessary to perform the technique(s) plus the corresponding dead volume.

Although they are not sterile products, microbiological contamination is controlled, although not monitored since the solutions contain sodium azide as a bacteriostatic and bactericidal agent. This additive improves device performance without affecting measurement results.

7.2 Application

Immunohistochemical determinations on paraffin-embedded tissue

7.3 Storage conditions

Fridge or cold store between 2 and 8 °C. Do not freeze.

7.4 Validity period

The device, if preserved in the established storage conditions, can be used up to the expiration date indicated on the label, even if opened. If the reagent has been stored in different conditions than the ones specified, the user must make sure that it works correctly, being aware that the device warranty is no longer valid.

7.5 Special instructions for manipulation

This kit is specially designed for its handling in the immunostainers MD-Stainer.

8 ANALYTICAL PERFORMANCE CHARACTERISTICS

8.1 Calibrators and control materials

The specifications of calibrators and control materials will be determined by the primary antibody used. See Instructions for Use for the specific primary antibody to be developed with the kit Master Polymer Plus Detection System (Peroxidase).

8.2 Measurement range, which is valid for any of the immunohistochemical procedures used:

Ideally, and always depending on the primary antibody used, the immunostaining obtained should be assessed at least by means of the semiquantitative procedures previously designed for immunohistochemical studies. These procedures must be implemented by each client laboratory. Among them, those that adopt valuation systems based on at least five degrees of intensity are more recommendable.

Manual quantification of immunostaining obtained by percentage count of positive cells is highly recommended as an alternative to semiquantitative assessment, and even better, its quantitative assessment by automated morphometry and/or computational pathology systems if they have been validated and enabled in the client laboratory.

9 PRODUCT LIMITATIONS

Vitro SA has optimized the kit Master Polymer Plus Detection System (Peroxidase) for use with reagents manufactured by the company. Each laboratory should use its own primary antibodies and should follow the recommended assay procedures. If they are not followed, they will accept responsibility for the interpretation of patient results in these circumstances.

If the user uses concentrated antibodies, the appropriate dilution of primary antibodies should be determined empirically as it may vary due to changes in tissue binding and antigen stimulation efficacy. Negative reagent controls should be used to improve recovery conditions and primary antibody concentrations.

The use on frozen tissue has not been evaluated. For other limitations see sections 6 and 8, as well as the corresponding sections in the instructions for use of the specific primary antibody manufactured by Vitro SA, which you wish to develop with the kit Master Polymer Plus Detection System (Peroxidase).

10 SAMPLE TYPES AND TARGET TEST POPULATION

Paraffin-embedded tissue sections at 4 microns thickness and mounted on special slides for immunohistochemistry. These sections do not require any special conditions for sample collection, handling and preparation other than the standards established in each laboratory, except in the case of suspected prion infection where appropriate preventive measures should be taken (see also section 13). In buffered formalin-fixed samples the determination is direct and universal. If the sample has been processed in other fixatives or if it is frozen material, a local validation of the procedure must be carried out beforehand. Product intended for people of any age range requiring the analysis of the expression levels of certain molecules by immunohistochemical techniques on formalin-fixed and paraffin-embedded tissue.

11 ANALYTICAL PRINCIPLE OF THE METHOD

Antigen detection on tissues and cells using a multistep immunohistochemical procedure that at least includes incubation with the primary antibody, incubation with an enzymatically labeled bridging immune reagent (ideally multispecies and polymeric in structure), revealing of such activity by a colorimetric reaction, contrast staining with hematoxylin and corresponding washes between steps.

For the recommended incubation time for the primary antibody, antigenic retrieval protocol, as well as other details, refer to the protocols preloaded on the MD-Stainer. It is recommended to follow the standard procedures of each laboratory, refer to the instructions recommended by the manufacturer of each product and follow the procedures programmed on the immunostainer.

12 RECOMMENDED WORKING PROTOCOL

12.1 Sample preparation (for paraffin-embedded tissues)

The sample can undergo an antigen denaturation if it undergoes to a prolonged fixation. Therefore, and in order to obtain an optimal fixation with the tissue maintaining its antigenic activity, it is recommended to fix it with 10% buffered formalin for 24-48 hours.

12.2 Section preparation (for paraffin-embedded tissues)

Sections are cut at 3 µm and placed on the slide. If there is a need to do more treatments as an antigen retrieval, through heat or enzymatic treatment, the glass slide must be covered with a sticker for tissue sections as 0.02% poly-L-lysine or silane.
It is recommended to use a tissue sample with positive immunoreactivity and a negative one for the primary antibody used, or replace the primary antibody with washing buffer or normal serum and process them the same way as the template sample for a correct interpretation of the staining results.

12.3 Antigen retrieval and technical protocol

When using the MD-Stainer, the best results are obtained when using the unmasking protocol established by the supplier and preloaded on the instrument for each primary antibody.

12.4 General Notes

1. The recommendations for use included in this data sheet are general. It is recommended to perform the technique with the routine processes of each laboratory.
2. When cleaning, it must be avoided to use detergents containing hypochlorite.
3. For a correct use of the results, it is recommended to always use positive and negative controls and, eventually, isotypic controls in the case of some monoclonal antibodies.
4. Use the slides treated with chromogel, poly-L-lysine or 3-amino-propyl thrietoxysilane.
5. It is recommended to use water purified through reverse osmosis with conductivity greater than 10 megaohms.
6. In order to decrease the denaturation of the antigen, it is recommended to use paraffins with a low melting point (<60 °C).
7. The tissue antigens will be preserved better if they are fixed through a newly prepared 4% paraformaldehyde buffer.
8. The use of this Kit with the automatic system MD-Stainer requires the use of the technical conditions recommended by the supplier.

12.5 Causes of excessive staining

1. The endogenous peroxidase has not been blocked.
2. Incomplete deparaffinization of the sections.
3. Excess of adherents for the tissue on the slides.
4. Incorrect dilution of the primary antibody.
5. Low-quality water or containing hypochlorites.
6. Use of impure DAB.

12.6 Causes of absence of staining

1. The primary antibody or the amplifying complex of primary antibodies have not been applied.

2. Inadequate fixation with excessive antigen retrieval.
3. Errors in the preparation of the DAB solution.

13 EQUIPMENT AND PRODUCTS REQUIRED

13.1 Supplied with the reagent:

The following reagents are supplied with the kit:

- Peroxidase Blocking Reagent
- Primary Antibodies Amplifier Master
- Master Polymer Plus HRP
- DAB Substrate
- DAB Chromogen
- DAB Enhancer
- Contrast Hematoxylin HDH3

13.2 Not supplied with the reagent

Reagents, materials and equipment necessary for the immunohistochemistry technique are available in the VITRO S.A. product catalog, but not supplied with the device:

- Primary antibodies
- MD-Stainer
- Pretreatment Kit
- Silane-treated slide
- TBS buffer – Tween 20
- Optical microscope and/or a digital scanner of histological slides

All the immunostaining process of the sections and the codes of the system (which allows the online recognition of the reagent and the slides undergoing a study) are programmed in the immunostainer's software. Therefore, when a laboratory implements this analysis procedure for the first time, it is essential to ensure that the information accumulated in the instrument is correctly programmed. Contact your Authorized Supplier / Distributor if required.

Keep in mind that the optimal working conditions can vary depending on the type of tissue and that, in any case, they must be individually established for each laboratory. In general and in order to optimize the process, we recommend the use of primary antibodies produced by Vitro S.A. and specific buffers and consumables for these instruments in the immunostainer.

If the product is to be used with different systems other than those recommended above, it is necessary to carry out a prior validation process in each laboratory.

14 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.** When the product is used in conjunction with a primary antibody as an aid to diagnosis, or to establish parameters of prognostic or predictive value about neoplasms, it should only be handled by trained users and in authorized laboratories and strictly following the instructions contained in this brochure.
- **Optimization and interpretation.** It should be borne in mind that the ultimate responsibility for the optimization and interpretation of the immunostaining performed lies with the physician and technicians who use it and that, likewise, this reagent is only a tool for the interpretation of the morphological findings of each case in conjunction with other diagnostic and prognostic tests and the pertinent clinical data of the

patient.

- **Use:** Except for the requirement that the special slides on which the sections to be treated with this reagent are mounted (which are electrostatically charged) must be kept away from sources of radiation, and those relating to exposure to high temperatures or excessive light, there are no other external or environmental influences on the reagent or interference of the reagent with other investigations or treatments to be performed on the patient.
- **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 each component 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.
- **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"
Perishable material (tubes, tips, aluminum foil, 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, hematoxylin, alcohol, eosin), generated from immunostaining techniques.	200121	"Solvents"

Table 3. Classification of wastes 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 materials.**

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16 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		Sufficient content for <n> assays
	Safety data sheet		

17 CHANGELOG

Date	Description
2022-10-17	Creation of the document