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) (Incl. DAB Chromogen)

Table of contents

1 CATALOG REFERENCES AND PRESENTATIONS

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

Reference	Components of the Kit						
	No. of Tests	Peroxidase Blocking Reagent	Primary Antibody Amplifier	Polymer	DAB substrate buffer	DAB chromogen	DAB enhancer
MAD-000237QK	500	50 mL	50 mL	50 mL	60 mL	2 mL	50 mL
MAD-000237QK-10	100	10 mL	10 mL	10 mL	15 mL	0.5 mL	10 mL
MAD-000237QK-100	1 000	100 mL	100 mL	100 mL	150 mL	5 mL	100 mL
MAD-000237QK-125	1 250	125 mL	125 mL	125 mL	180 mL	6 mL	125 mL
MAD-000237QK-1L	(1)	1000mL	1000mL	1000mL	1200mL	50mL	1000mL

(1) Bulk format (Number of test according to use)

2 INTENDED PURPOSE OF THE PRODUCT

The product Master Polymer Plus Detection System (Peroxidase) (Incl. DAB Chromogen) 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 and manual immunohistochemistry 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) (Incl. DAB Chromogen) 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

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

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.

Positive and negative results obtained using antibodies developed with the Master Polymer Plus Detection System (Peroxidase) (Incl. DAB Chromogen) 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.

Although this product has been designed for manual and automated handling in LabVision Autostainer immunostainers, it can also be used for immunohistochemistry in other automated immunohistochemical staining systems than the recommended one, although it is highly recommended that the procedure be subjected to close review and/or eventual validation by each laboratory.

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) (Incl. DAB Chromogen).

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) (Incl. DAB Chromogen).

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) (Incl. DAB Chromogen).

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) (Incl. DAB Chromogen).

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) (Incl. DAB Chromogen).

7 DEVICE DESCRIPTION

7.1 Composition

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

Description	References
Peroxidase Blocking Reagent	MAD-021540Q-10, MAD-021540Q-50, MAD-021540Q-100M, MAD-021540Q-125 or MAD-021540Q-1L
Primary Antibodies Amplifier Master	MAD-000237QK-B10, MAD-000237QK-B, MAD-000237QK-B100M, MAD-000237QK-B125 or MAD-000237QK-B1L
Master Polymer Plus HRP	MAD-000237QK-C10, MAD-000237QK-C, MAD-000237QK-C100M, MAD-000237QK-C125 or MAD-000237QK-C1L
DAB Substrate Buffer	MAD-001812QK-A, MAD-001811QK-A, MAD-001811QK-A100M, MAD-001811QK-A125 or MAD-001811QK-A1L
DAB Chromogen Concentrate	MAD-001812QK-B, MAD-001811QK-B, MAD-001811QK-B100M, MAD-001811QK-B125 or MAD-001811QK-B1L
DAB Enhancer	MAD-001560Q-10, MAD-001560Q-50, MAD-001560Q-100M, MAD-001560Q-125 or MAD-001560Q-1L

All components are supplied in liquid form and in ready-to-use format except DAB Chromogen Concentrate which is supplied in concentrated format.

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 manual handling in LabVision Autostainer immunostainers, although its use in other automatic immunostainers and manual procedures is possible as long as the reagent is validated locally according to the conditions existing in each laboratory.

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) (Incl. DAB Chromogen).

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) (Incl. DAB Chromogen) 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 7 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) (Incl. DAB Chromogen).

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, see Instructions for Use for the specific primary antibody to be developed with the kit Master Polymer Plus Detection System (Peroxidase) (Incl. DAB Chromogen). 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

The sections must be subjected to heat-induced antigen retrieval according to the standard procedure of each laboratory. In short, the process includes deparaffinization of the sections, heat-induced antigen retrieval and subsequent washes in buffer.

The details of antigenic retrieval 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) (Incl. DAB Chromogen).

Although this product has been successfully used in immunosorbent assays other than those mentioned above, its use in them requires prior validation.

12.4 Deparaffinization and Hydration

1. Deparaffinization with xylene.
2. Hydration of the samples with decreasing alcohols.
3. Hydration with bidistilled or deionized water.

12.5 Staining procedure

12.5.1 Peroxidase Blocking Reagent

- Apply 100 µL of **Peroxidase Blocking Reagent** to each of the samples to completely cover the sections. Incubate at room temperature for **10 minutes** at room temperature and in the dark.
- Rinse the slides in TBS 3 times for 5 minutes.

12.5.2 Incubation of the primary antibody

- Coat the test tissue section following the recommendations given by the manufacturer for the use of that antibody.
- Rinse in TBS 3 times for 5 minutes.

12.5.3 Incubation with the Primary Antibody Amplifier

- Apply 100 µL of **Primary Antibodies Amplifier Master** to each of the samples to completely cover the sections. Incubate at room temperature for **15 minutes**.
- Rinse in TBS 3 times for 5 minutes

12.5.4 Incubation with the Polymer

- Apply 100 µL of **Master Polymer Plus HRP** to each of the samples to completely cover the sections. Incubate at room temperature for **30 minutes.**
  Note: The micropolymer is sensitive to light. Avoid unnecessary exposure to light and store in an opaque vial or container.
- Rinse in TBS 3 times for 5 minutes.

12.5.5 Immunostaining development (Preparation and incubation with substrate/chromogen)

Preparation of the substrate/chromogen mix: Add **1 drop** of concentrated **DAB Chromogen Concentrate** to **1 ml** of **DAB Substrate Buffer**. Mix well. This solution should be kept away from light. Under these conditions the solution is stable for hours.

- Apply the substrate/chromogen mix to each sample until the sections are completely covered.
- Incubate at room temperature for **5 minutes**.
- Wash with distilled water 3 times for 5 minutes.

12.5.6 Staining intensification (this step is not mandatory)

- Apply 100 µL of DAB Enhancer to each of the samples to completely cover the sections. Incubate at room temperature for **2 minutes.**
- Rinse with distilled water 3 times for 5 minutes.

12.5.7 Sample contrast

- Cover the specimen with hematoxylin for contrast staining for 1-2 min.
- Wash well with bidistilled or deionized water.

12.5.8 Rinsing and mounting

- After washing with water, dehydrate in successive steps in alcohol of increasing alcohol content, rinse and mount with permanent mounting medium.

12.6 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 triethoxysilane.
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 Pathcom slide stainer 1 requires the use of the technical conditions recommended by the supplier.

12.7 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.

12.8 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 Buffer
- DAB Chromogen Concentrate
- DAB Enhancer

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
- LabVision Autostainer
- 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.**

15 BIBLIOGRAPHY:

1. Banks, P. M. Diagnostic applications of an immunoperoxidase method in hematopathology. J. Histochem Cytochem 27(8): 1192- 1194, 1979.
2. DeLellis, R. A. Basic techniques of immunochemistr y, In: Diagnostic Immunohistochemistry , R. A. DeLellis, ed., Masson Publishing USA, New York, 1981, pp. 7- 16.
3. Elias, J. Immunohistopathology: A Practical Approach to Diagnosis. 2nd Ed., ASCP Press, Chicago, USA, 2003.
4. Taylor & Cote, Immunomicroscopy: A Diagnostic Tool for The Surgical Pathologist, 2nd Ed. Philadelphia, WB. Savnders Co. 1994.
5. Shi SR, Guo S, Cote RJ, Young L, Hawes D, Shi Y, Thu S, Taylor CR, Applied Immunohistochemistry & Molecular Morphology, vol 7, 201-208, 1999.
6. Shi SR, Cote RJ, Taylor CR. Antigen retrieval immunohistochemistry and molecular morphology in the year 2001. Appl Immunohistochem Mol Morphol. (2): 107-16. 2001.
7. National Committee for Clinical Laboratory Standards. Internal quality control testing: principles and definitions; approved guideline. Villanova, PA 1991; Order Code C24-A:4.
8. Escribano LM, et al. Endogenous peroxidase activity in human cutaneous and adenoidal mast cells. J Histochem Cytochem 1987; 35:213.
9. Omata M, et al. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen: A possible source of error in immunohistochemistry. Amer J Pathol 1980; 73:626.
10. Tubbs RR, et al. Atlas of Immunohistology. Chicago: Amer Soc Clin Pathol Press 1986.
11. Nadji M and Morales AR. Immunoperoxidase techniques, a practical approach to tumor diagnosis. Chicago: Amer Soc Clin Pathol Press 1986.
12. Cartun RW. Immunohistochemistry of infectious diseases. J Histotechnol 1995; 18(3):195.
13. Heras A, et al. Enhanced labelled-polymer system for immunohistochemistry. XVth Eur Cong Pathol. Copenhagen, Denmark 1995; Sept 3-8.
14. Bisgaard K and Pluzek K-P. Use of polymer conjugates in immunohistochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates. Abstract. XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungary 1996; Oct 20-25.

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.
2023-08-29	References for new 1L format are included in sections “1. Catalog references and presentations” and “7.1 Composition”
2023-09-07	Explanatory note is included in Section “1. Catalog references and presentations”