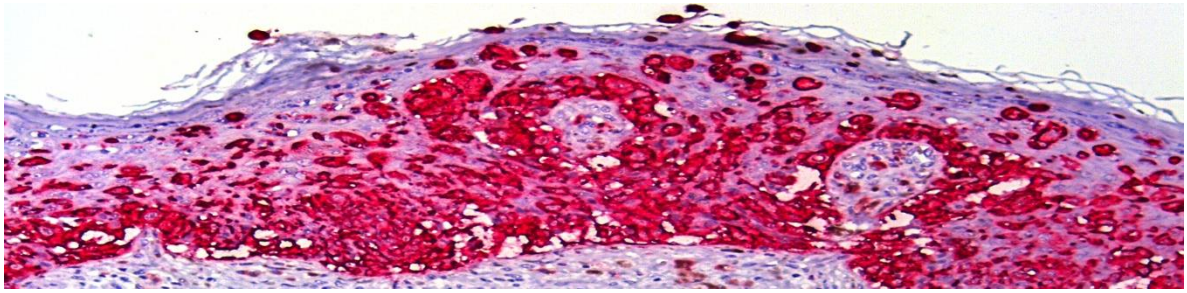
VITRO, S.A
Calle Luis Fuentes Bejarano, 60
Edificio Nudo Norte, Local 3
41020 Sevilla (España)
F: +34 954 922 892
T: +34 954 933 200
www.vitro.bio

MASTER POLYMER PLUS DETECTION SYSTEM (PHOSPHATASE) (INCL. AP CHROMOGEN)

MASTER POLYMER PLUS ALKALINE PHOSPHATASE DETECTION SYSTEM
ALKALINE PHOSPHATASE – (AP)



PRESENTATION:

The sale presentations for this product are the following[1]:

Kit/reference	Amplifier Acs. primary Master - AP	Polymer Master Plus-AP	Substrate buffer AP	Chromogen AP
MAD-000230QK	50 mL	50 mL	2x60 mL	2 mL
MAD-000230QK-10	10 mL	10 mL	2x15 mL	0,5 mL
MAD-000230QK-1L	1000 mL	1000 mL	3x800 mL	40 mL

SPECIFICITY, INTERFERENCES AND LIMITATIONS:

Master Polymer Plus AP is the result of the experience of a decade in systems of polymers for immunohistochemistry. The detection system has proven greater sensitivity with an increase in the antigen-antibody binding signal. The new technology of micropolymers provides greater advantages as compared to the conventional immunohistochemistry techniques and facilitates the detection of antigens in the different cellular compartments (nucleus, cytoplasm, cytoplasmic membrane) due to the smaller size of the molecule.
The technical procedure used eliminates the background staining originated by the non-specific binding to endogenous biotin molecules, as **Master Polymer Plus AP** is a procedure not based in the reaction avidin/biotin.
The detection system **Master Polymer Plus AP** is highly sensitive, provides a low background staining and the results obtained are higher than the ones obtained with the conventional procedures of Streptavidin/Biotin or long polymers.

[1] Product of use in *in vitro diagnostic*

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The system **Master Polymer Plus AP** is elaborated with the technology of micropolymers.

This system can be used with:
- Monoclonal primary antibodies obtained in mouse.
- Monoclonal and polyclonal primary antibodies obtained in rabbit.

APPLICATION OF THE PRODUCT:

This Kit is developed for immunohistochemical study using monoclonal or polyclonal **primary antibodies.** After incubating the selections with the unmarked primary antibody, the universal amplifier of primary antibodies is applied, followed by a complex constituted by micropolymer with universal secondary antibody and molecules of alkaline phosphatase. After the predetermined incubation times and after washing, it is developed using a specific chromogen of Alkaline Phosphatase (AP) type or the reagents of the developed kit.

KIT COMPONENTS:

MAD-000230QK (500 Test)	**MAD-000230QK-10 (100 Test)**	**MAD-000230QK-1L (*Bulk* Format)**	
MAD-000230QK-B	MAD-000230QK-B10	MAD-000230QK-B1L	Primary Antibodies Amplifier Master AP
MAD-000230QK-C	MAD-000230QK-C10	MAD-000230QK-C1L	Master Polymer Plus AP
MAD-001815QK-A	MAD-001818QK-A	MAD-001815QK-A1L	AP Substrate Buffer
MAD-001815QK-B	MAD-001818QK-B	MAD-001815QK-B1L	AP Chromogen Concentrate

REAGENTS AND ACCESSORIES NEEDED NOT PROVIDED WITH THE KIT:

1) Reagents: Reagents for the deparaffining and antigenic recovery for each antibody, primary antibodies, washing buffers and bi-distilled water (distilled deionised water or equivalent).

2) Accessories: Microscope, slide and coverslip for microscopes, pipettes, sample tubes, humid hatchery chamber and media mounting.

RECOMMENDATIONS FOR USE:

SAMPLE PREPARATION (for paraffin-embedded tissues)

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

SECTIONS PREPARATION (for paraffin-embedded tissues)

The sections are cut at 3µm and placed on the slides. If there is a need to do more treatments as antigenic recovery, through heat or enzymatic treatment, the crystal slides must be covered with a sticker for tissue sections as 0.02% poly-L-lysina or silane.
It is recommended to use a tissue sample with positive immunoreactivity and another one negative, or substitute 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.

SECTIONS PROCESSING (for paraffin-embedded tissues)

A. DEPARAFFINING AND HYDRATION

1. Deparaffining with xylene.
2. Hydration of the samples with decreasing alcohols.
3. Hydration with bi-distilled or deionized water.
4. Antigenic recovery with heat or enzymatic recovery according to the conditions recommended by the supplier, specific for each primary antibody

Note: if the antigenic recovery is done through heat, all these steps/solutions can be substituted with the use of the "antigenic recovery buffers" which facilitate deparaffining, hydration and antigenic recovery simultaneously.

B. STAINING PROCEDURE:

1. Incubation with the primary antibody
- Covering the template tissue section following the recommendations provided by the manufacturer for the use of this antibody.
- Clearing in TBS 3 times for 5 minutes.

2. Incubation with the amplifier of primary antibodies Master AP:
- Applying two drops (100 µL) of the Amplifier **(Master Polymer Plus AP)** to each one of the samples to completely cover the sections. Incubating at room temperature for **10 minutes.**
- Clearing in TBS 3 times for 5 minutes

3. Incubating with the Polymer Master Plus AP:
- Applying two drops (100 µL) of the micropolymer **(Master Polymer Plus AP)** to each one of the samples to completely cover the sections. Incubating at room temperature for **15 minutes**.

Note: The micropolymer is sensitive to the light. Avoid the unnecessary exposure to light and store in an opaque vial or container.
- Clearing in TBS 3 times for 5 minutes.

4. Developing of the immunostaining (preparation and incubation with substrate/chromogen)
The mix substrate/chromogen must be preparedin the moment in which it is needed or, maximum, 30 minutes earlier.

Adding **1 drop** of **red Chromogen AP** concentrated at **2.5 ml** of **substrate buffer** (or 2 drops at 5ml). Mix well. This solution must be safeguarded from light.

- Applying the mix substrate/chromogen to each one of the samples until completely covering the sections.
- Incubating at room temperature for **15 minutes**.
- Washing with distilled water 3 times for minutes.

5. Sample contrast
- Covering the sample with haematoxylin for contract staining, for **1-2 min according to the intensity of the desired contrast**.
- It is recommended not to use alcoholic solutions of contrast haematoxylin
- Washing properly with bi-distilled or deionized water.

6. Clearing and mounting
- After washing with water, **drawing the slides at 50-60ºC for at least 30 minutes or 1 hour at room temperature**, **clearing in xylene and install with permanent mounting medium.**

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GENERAL NOTES:

1. The recommendations of use included in this data sheet are general. It is recommended to perform the technique with the routine processes of each laboratory.
2. In the cleaning, we must avoid using detergents containing hypochlorite.
3. For a correct use of the results 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. Do not allow the sections to be drawn during the incubations.
6. Delete the greatest possible amount of buffer after the washings.
7. It is recommended to use water purified through inverse osmosis with conductivity greater than 10 megaOhms.
8. In order to decrease the denaturalization of the antigen, it is recommended to use paraffins with a low melting point (<60ºC).
9. The tissue antigens are preserved better if they are fixed through a newly prepared 4% paraformaldehyde buffer.

Causes producing a staining excess
1. Incomplete deparaffining of the sections.
2. Excess of adherents for the tissue on the slides.
3. Incorrect dilution of the primary antibody.
4. Water of low quality or hypochlorite content.
5. Use of impure chromogen.

Causes producing lack of staining
1. Not applying the primary antibody or the amplifying complex of primary antibodies.
2. Destruction of the antigen during the protocol development.
3. Inappropriate fixing with excessive antigen masking.
4. Use of azide in the washing solutions.
5. Allowing the sample to be dried during some step of the protocol.
6. Errors in the preparation of the chromogen solution of use.

Other causes producing a weak staining
1. Not draining the washing solutions properly before adding the immunological reagents.
2. Using wrong dilutions of the primary antibody.
3. Excessively prolonging the time between the preparation and use of the substrate solution of the chromogen.

STORAGE:

Store refrigerated until the expiration date (between 8ºC and 2ºC).

SAFETY RECOMMENDATIONS

The product is exclusively thought for a professional use in a laboratory, and not as a drug, for domestic use or other purposes. The current version of the Safety Data Sheet of this product can be downloaded searching for its reference at www.vitro.bio or can be requested at regulatory@vitro.bio.

BIBLIOGRAPHY:

- Shan-Rong Shi,James Guo,Richard J.cote,Lillian Young, Debra Hawes, Yan Shi, Sandra Thu and Clive R.Taylor, Applied Immunohistochemistry & Molecular Morphology, vol 7,201-208,1999
- DeLellis, R. A. Basic techniques of immunochemistr y, In: Diagnostic Immunohistochemistry , R. A. DeLellis, ed., Masson Publishing USA, New York, 1981, pp. 7- 16.
- Elias, J. Immunohistopathology: A Practical Appr oach to Diagnosis, ASCP Press, Chicago, USA, 1990.
- Taylor & Cote, Immunomicroscopy: A Diagnostic Tool For The Surgical Pathologist, 2nd Ed. Philadelphia, WB. Savnders Co. 1994.
- 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.
- Shi SR, Cote RJ, Taylor CR. Antigen retrieval immunohistochemistry and molecular morphology in the year 2001. Appl Immunohistochem Mol Morphol. (2): 107-16. 2001.

LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

IVD	Health product for in vitro diagnosis.		Expiration date
REF	Catalog number		Temperature limit
LOT	Lot code		Manufacturer
	Refer to the instructions of use		Sufficient content for <n> assays
	Material safety data sheet		