


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 DUAL STAINING KIT

Description: Master Dual Staining Kit contains reagents to perform manual or automated immunohistochemistry dual staining on human tissue sections, fixed in buffered formalin and embedded in paraffin.

This kit contains a visualization system based on micro polymers with the following presentations:

Presentation ▽: The sale presentations for this product are the following:

Reference	No. of Tests	Peroxidase Blocking Reagent	POLYMER MIX	DAB Substrate Buffer	DAB Chromogen Concentrate	AP Substrate Buffer	AP Chromogen Concentrate	DAB Enhancer
¹ MAD-001882QK	100	10 mL	10 mL	15 mL	0,5 mL	2x15 mL	0,5 mL	10 mL
MAD-001882QK-1L	² N.A.	1000 mL	1000 mL	2x750 mL	50 mL	3x1000 mL	50 mL	1000 mL

(1) These references are for presentation in vials of Low Density Polyethylene (LDPE) dropper. In case the products are used in LabVision AutoStainer, a special reference is assigned as follows:


- / L: Cylindrical screw-cap vials (QD-3 / L, QD-7 / L, QD-12 / L).
- / N: Polygonal screw-cap vials (QD-3 / N, QD-7 / N, QD-12 / N).

(2) Bulk format. Number of test according to use.

For different presentations (references / volumes) please contact the supplier.

Intended Use : Diagnostic in vitro in humans

Storage conditions : refrigerator between 2 and 8 ° C.

Warranty : The container once opened, the reagent can be used until the expiration date indicated on the label. If the reagent has been stored under conditions other than those indicated in this document, the user must previously check its correct functionality considering that the product's warranty is no longer valid.

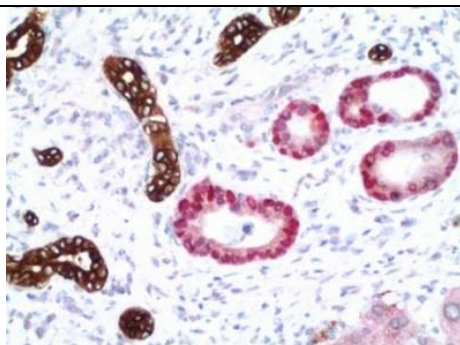
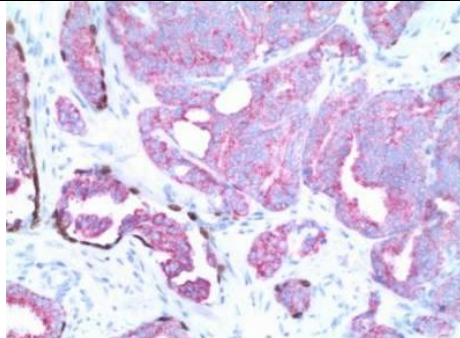
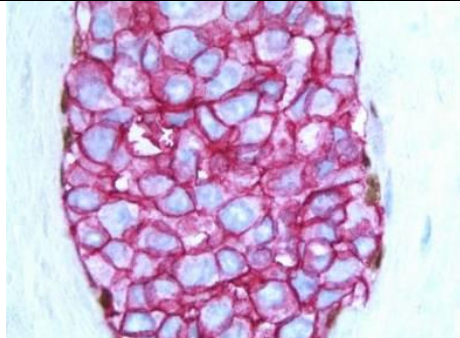
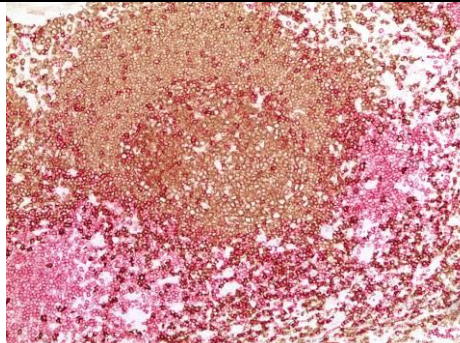
Special Handling Instructions: This reagent is specially designed for handling in Lab Vision Autostainers or manual handling.

Warnings and Precautions: 1) The product may only be operated by trained users and authorized laboratories.

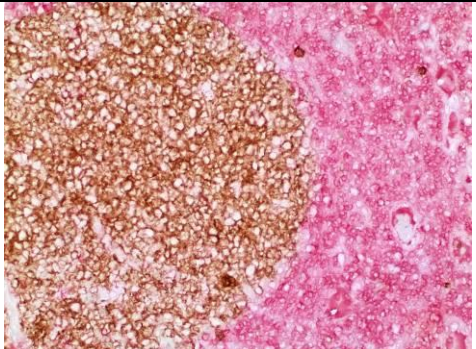
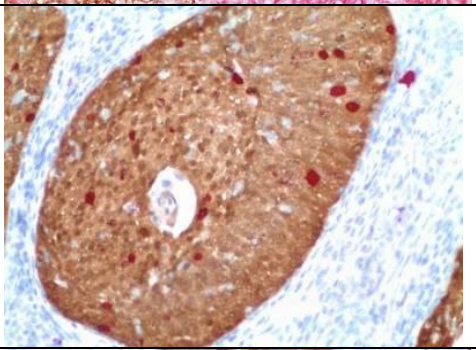
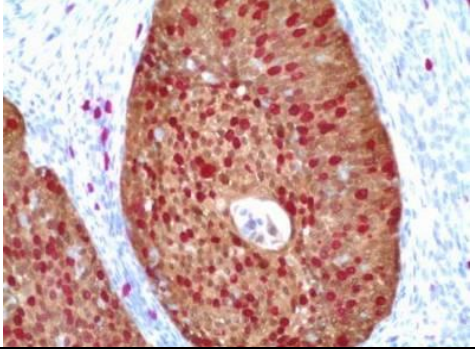
2) Please note that the ultimate responsibility in the optimization and interpretation of chromogenic hybridizations technique corresponds to the attending physician and technicians who use the kit. Also, this set of reagents is only a useful tool for the interpretation of morphological findings of each case in conjunction with other relevant diagnostic tests and patient's clinical data.

3) The reagent contains sodium azide (NaN₃) as a preservative. Although this product is highly toxic and if mixed with water or acids, mainly in the presence of metals there is danger of explosion, these risks are minimized to the maximum when used at concentrations below 0.05% as in this case. However, for handling this reagent the following precautions should be taken: a) Use of gloves and protective equipment established for hybridization and immunohistochemistry techniques and lab strict compliance with the general safety practices existing in it; b) Do not store reagents in metal packaging and do not use metal tools for its handling c) Store waste for disposal in appropriate containers regulated under current regulations in each laboratory.

POSSIBLE COMBINATIONS OF ANTIBODIES TO BE USED WITH MASTER DUAL STAINING KIT¹:

Mix of Antibodies	Use	Photo
CK7+CDX2 (MAD-001004QD+ MAD-000645QD)	Possible use in differentiating metastatic tumor of intestinal differentiation from a tumor originating in the lung, pancreas, breast or ovary.	
p504 (racemase)+p63 (MAD-000485QD)	PIN cocktail - differentiate areas of normal prostate, PIN and prostate acinar adenocarcinoma.	
p63+C-erbB2 (MAD-000479QD+ MAD-000308QD)	Simultaneous assessment of HER2 protein expression in both, the in situ and the infiltrative components.	
CD20+CD3 (MAD-002037QD+ MAD-000621QD)	Differentiate the B and T components of a lymphoid proliferation.	

¹ The Mix of Antibodies are not included in the kit

<p>CD10+Bcl2 (MAD-002022QD+ MAD-00675QD)</p>	<p>Generally, differentiate between the benign lymphoid germinal center from a malignant follicular proliferation of B lymphocytes.</p>	
<p>p16INK4a +PHH3 (MAD-000690QD+ MAD-000584QD)</p>	<p>Identify mitosis in a dysplastic cervical epithelium.</p>	
<p>p16INK4a+Ki67 (MAD-000690QD + MAD-000310QD)</p>	<p>Evaluation of the mitotic index in a cervical dysplastic epithelium. Useful in the evaluation of cervical cytological smears.</p>	

LIMITATIONS OF THE REACTIVES

The use on frozen tissue has not been evaluated.

In order to use the Master Dual Staining kit **it should be noted that the two antibodies must come from different animals (mouse / rabbit).**

Due to the presence of a soluble chromogen in alcoholic solutions, the slides must be air-dried and for final mounting an aqueous mounting media should be used.

SAMPLE TYPES

Sections of 4 microns thick mounted on special slides for immunohistochemistry and obtained from paraffin-embedded tissues, preferably fixed in buffered formalin.

PRINCIPLE OF THE ANALYTICAL METHOD

The aim of the immunohistochemical staining is to convert into a visible stain the resulting antigen-antibody unit to be studied in cells or tissues.

Thus, the Master Dual Staining Kit provides a set of highly specific and sensitive reagents which allow dual staining of two different antigen-antibody units.

The kit is based on the use of a mix of two micropolymers, each labeled with different enzymes (Peroxidase and Alkaline Phosphatase) each recognizing a specific immunoglobulin (antibody) developed in rabbit and mouse respectively.

In case the reaction has occurred between the primary antibodies and antigens, the micropolymers specifically bind to the resulted antigen-antibody unit. Due to enzyme label of the polymers, adding chromogen and its corresponding substrates, precipitates of different colors are obtained which allows to microscopically detect the presence of a specific antigens.

In the case of Master Dual Staining Kit, brown precipitates will be obtained for the antibodies developed in mouse and red / purple color for rabbit antibodies.

COMPONENTS AND REAGENTS INCLUDED IN THE KIT:

MAD-001882QK (100 Test)	MAD-001882QK-1L	
MAD-021540Q-10	MAD-021540Q-1L	Peroxidase Blocking Reagent
MAD-001882QK-C	MAD-001882QK-C1L	POLYMER MIX
MAD-001812QK-A	MAD-001811QK-A1L	DAB Substrate Buffer
MAD-001812QK-B	MAD-001811QK-B1L	DAB Chromogen Concentrate
MAD-001818QK-A	MAD-001815QK-A1L	AP Substrate Buffer
MAD-001818QK-B	MAD-001815QK-B1L	AP Chromogen Concentrate
MAD-001560Q-10	MAD-001560Q-1L	DAB Enhancer

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT:

- PT module or humid incubation chamber
- PT module buffers
 - CITRATE (**MAD-004071R/D**)
 - EDTA (**MAD -004072R/D**)
 - TRIS-EDTA (**MAD-004070R/D**)
- Slides treated with silane or electrically charged (**MAD-15-188-55/100**)
- Coverslips
- Thermal plate or oven (37 ° C)
- Dewaxing and hydration battery (xylene, absolute ethanol and of 80% and 70% concentrations)
- TBS buffer (**MAD-004077R-10**)
- Micropipettes
- Contrast hematoxylin (**MAD-108.1000**)
- Optical microscope
- Mix of Antibodies

TECHNICAL PROTOCOL FOR DOUBLE IMMUNOHISTOCHEMICAL STAINING USING MASTER DUAL STAINING KIT

1. Dewaxing and heat induced antigen retrieval²

- Incubate slides with paraffin tissue sections in the oven at 60 ° C overnight.
- Place the slides in the PT module using the flowing conditions:
 - Pre-heat at 65°C
 - Dewax than followed by antigen retrieval 20 minutes a 95°C

2. Detection and visualization (manual or automatic)

- Wash in TBS - Tween 20 at RT
- Apply **100 µl of Peroxidase Blocking Reagent** on the tissue and incubate 10 min at RT
- Wash 3 times in TBS.
- Apply **100 µl of 1° Antibody*** and incubate for 10 minutes** at RT
- Wash 3 times in TBS.

² For dewaxing, tissue rehydration and antigen retrieval, alternative procedure could be used

- f. Apply **100 µl of 2º Antibody*** and incubate for 10 minutes** at RT
- g. Wash 3 times in TBS.
- h. Apply **100 µl of POLYMER MIX** and incubate for 30 minutes at RT.
- i. Wash 3 times in TBS.
- j. Mix a **drop of DAB Chromogen Concentrate** with **1 ml of DAB Substrate Buffer** and applying the resulting solution onto the tissue; incubate for 5 min at RT
- k. Wash 3 times in distilled water
- l. Mix a **drop of AP Chromogen Concentrate** with **2,5 ml of AP Substrate Buffer** and applying the resulting solution onto the tissue; incubate for 10 min at RT
- m. Wash 3 times in distilled water

Note: DAB marking can be powered using our DAB Enhancer (MAD-001560Q), not supplied in the kit.

3. Contrast staining and mounting

- a. Stain with contrast hematoxylin.
- b. Bluing in tap water.
- c. Dehydrate and clear with increasing concentrations of alcohols and xylene.
- d. Mount and interpret the results under a microscope.

*** If the two antibodies are available as cocktail, incubation of the mixture will be done in one step.**

**** The final incubation time should be determined by the individual laboratory based on the mix of antibodies to be used.**

INCIDENTS AND COMPLAINS

It is recommended to thoroughly follow all instructions contained in these technical data sheet. In case of occurrence of atypical or unexpected results please contact the Vitro SA sales representative of the area. If not, please contact Vitro S.A SL using its contact information as mentioned above.

SAFETY RECOMMENDATIONS

This product is intended for laboratory professional use only. The product is NOT intended to be used as a drug or for domestic purposes. The current version of the Safety Data Sheet for this product can be downloaded by searching the reference number at www.vitro.bio or can be requested at regulatory@vitro.bio.

LIMITATIONS OF THE REACTIVE










If you have met all the conditions of storage and handling in the laboratory, this reagent is guaranteed throughout its warranty life. Vitro S.A SL is not responsible for damage, personal injury or economic loss that this reagent can be involved.

REFERENCES

1. Xiao Chen, Dan-Bi Cho, Ping-Chang Yang. Double staining immunohistochemistry. N Am J Med Sci. 2010 May; 2(5): 241–245.
2. Chris M. van der Loos. Multiple Immunoenzyme Staining: Methods and Visualizations for the Observation With Spectral Imaging. J Histochem Cytochem. 2008 April; 56(4): 313–328.
3. T. van Agthoven, M. Timmermans, J. A. Foekens, L. C. Dorssers, S. C. Henzen-Logmans. Differential expression of estrogen, progesterone, and epidermal growth factor receptors in normal, benign, and malignant human breast tissues using dual staining immunohistochemistry. Am J Pathol. 1994 June; 144(6): 1238–1246.
4. Ritu Bhalla, Lakshmi P Kunju, Scott A Tomlins, Kelly Christopherson, Connie Cortez, Shannon Carskadon, Javed Siddiqui, Kyung Park, Juan Miguel Mosquera, Gary Pestano, Mark A Rubin, Arul Chinnaiyan, Nallasivam Palanisamy. Novel Dual Color Immunohistochemical methods for detecting ERG-PTEN and ERG-SPINK1 status in prostate carcinoma. Mod Pathol. Author manuscript; available in PMC 2013 December 1. Published in final edited form as: Mod Pathol. 2013 June; 26(6): 835–848

LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

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