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CD10

MOUSE ANTI-CD10 MONOCLONAL ANTIBODY (Clone 56C6)

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1 CATALOG REFERENCES AND PRESENTATIONS

The references and presentations available for this product are described in the following table:

Table 1. References and presentation

Reference		Presentation ¹
MAD-002022	Q	1 mL
	QD-3	3 mL
	QD-7	7 mL
	QD-12	12 mL
	QDS-1	7 mL
	QDS-2	14 mL
	QDS-3	28 mL

¹ All product presentations are in Ready-to-Use format, except for the reference ending in Q, which is supplied in Concentrate format. References ending in S for use in the VitroStainer 42. The use of the product in other immunostainers must be validated locally according to the instrument and the existing conditions in each laboratory. Not to be used for western blotting and flow cytometry techniques.

2 INTENDED PURPOSE OF THE PRODUCT

CD10 is a primary antiserum for immunohistology for professional use, for the qualitative determination of CD10 antigen as an aid to *in vitro* diagnostics in the laboratory by automated and manual immunohistochemistry, on paraffin-embedded tissue in neoplasms and in all other clinicopathological and physiological situations where identification is required. The product is intended to be used in screening, diagnosis, staging or monitoring of cancer for people of any age with suspected neoplasms or other clinicopathological and physiological situations requiring the analysis of CD10 molecule expression.

This antibody is essential for the identification of clear cell renal carcinoma, endometrial stromal sarcoma and atypical fibroxanthoma and complementary to other markers to aid in diagnosis in certain lymphomas (follicular, large B-cell, etc.) and some carcinomas and sarcomas. The clinical interpretation of any staining or lack thereof should be supplemented by morphologic studies using appropriate controls and should be evaluated by a qualified pathologist in the context of the patient's medical history and other appropriate diagnostic tests and controls by a qualified pathologist.

3 OTHER POSSIBLE APPLICATIONS OF THE PRODUCT

This antibody has also been reported that CD10 is also of interest in the diagnosis of many other neoplasms, including germ cell neoplasms, choriocarcinoma, some vascular tumors such as epithelioid hemangioendothelioma, some gliomas, melanoma, prostatic and pancreatic adenocarcinoma, rhabdomyosarcoma and other sarcomas, including Ewing's sarcoma, schwannoma and mixed Müllerian tumors. A correlation has also been observed between CD10 expression levels in cancer and a higher histological grade, tumor size, vascular invasion and survival rate in patients with various solid tumors. Nevertheless, this antibody has not been validated for its use in these pathologies.

4 DESCRIPTION OF THE MOLECULE IDENTIFIED BY THE PRODUCT

The CD10 protein is a glycoprotein of the metallopeptidase group (zinc-dependent metalloendoproteases) of 90-110 kD of molecular weight genetically encoded on 2q21-27 and located on the cell surface. Its main function is to inactivate by hydrolysis the various peptide hormones of approximately 30 amino acids

(bombesin, opioids, enkephalins, angiotensins, natriuretic factor, etc.) that control normal cell^(1, 2) and tumor cell growth⁽³⁾. In neoplastic epithelial cells where CD10 is expressed, its loss of methylation leads to increased cell migration, growth and survival⁽⁴⁾. Alterations in the genomic sequence of neprilysin are linked to the spinocerebellar ataxia known as Charcot-Marie-Tooth disease type 2⁽⁵⁾.

Mammalian adaptive immunity depends on the specific recognition of exogenous aggressor antigens by thymus-generated and programmed T lymphocytes. This recognition is initiated when foreign peptides with antigenic capacity are identified by the alpha/beta chains of the T-cell antigen receptor (TCR) in the presence of the CD3 molecule, which acts as an efficient transducer to the cell nucleus of the signals generated by antigenic activation. For the recognition of exogenous peptides it is necessary that: 1) they are bound to a major histocompatibility complex (pMHC) displayed by some of the antigen presenting cells ((APC) in the tissues; 2) the participation as co-receptors of CD4 and/or CD8 molecules, which induce the necessary amplification of this first T cell activation signal until it becomes completely efficient, and 3) the triggering of a second T cell activation signal against the antigen, which in the case of helper T cells uses the CD28 molecule that induces the subsequent proliferation of the activated T cells, or CD70 and CD137 in the case of cytotoxic/suppressor T cells.

As a result of these primary and secondary processes, clonal amplification of T cells occurs until sufficient numbers of activated T lymphocytes are generated to stop invasion by exogenous agents. In this process, activated T cells receive survival-inducing signals from various T cell molecules (ICOS, CD137, OX-40, etc.) activated after interaction with the APC triggering TCR activation. Finally, the activated T-helper cells, depending on the cytokine environment (ILs) released by the remaining cells participating in the primary and secondary immune response, may eventually differentiate into Th1 (IL-12 induced), Th2 (IL4) or Th17 (IL6, IL23) effector cells, which act directly at the site of action against the antigen by secreting the cloud of cytokines that characterizes their corresponding responses.

Synonyms of the identified antigen: Neprilysin, specific and common acute lymphoblastic leukemia antigen (CALLA), atropeptidase, enkephalinase, neutral endopeptidase 24.11, skin fibroblast elastase.

5 EXPRESSION IN NORMAL TISSUES

Although the CD10 molecule was initially identified as a common acute lymphoblastic leukemia antigen (CALLA antigen)⁽⁶⁾ specific to these cells, its presence has subsequently been described on the surface of some non-neoplastic lymphoid cells, such as the early precursors of B-cells in the bone marrow and T-cells in the thymus⁽⁷⁾, maturing B-cells from lymphoid follicle centers^(2, 8), very few mature small B cells and a subpopulation of parafollicular T cells and bone marrow stem cells and some myelopoietic cells including granulocytes⁽⁹⁾.

In addition, CD10 is expressed on a wide variety of epithelial cells, such as the brush border of the gastrointestinal cells of the upper digestive tract⁽¹⁰⁾, the adrenal cortex⁽¹¹⁾, bile canaliculi of hepatocytes⁽¹²⁾, podocytes and the apical pole of tubular cells of the kidney⁽¹³⁾, alveolar cells of the lung, the genitourinary system, including secretory and basal cells of the prostate⁽¹⁴⁾, myoepithelial cells of the breast⁽¹⁵⁾, sweat glands of the skin and salivary glands and the epithelium of the Wolffian duct, not of Mullërian origin^(16, 17). In addition, CD10 stains some endothelia and fibroblasts as well as the cyto- and syncytiotrophoblast of the placenta^(18, 19) and the endometrial stroma⁽²⁰⁾, the mesonephric embryonic remains⁽²¹⁾ and some brain areas next to the choroid plexus⁽²²⁾.

6 DIAGNOSTIC, PROGNOSTIC AND PREDICTIVE AID APPLICATIONS

6.1 Diagnostic aid, monitoring and staging

6.1.1 CD10 in neoplasms of the hematopoietic system and with predominant membrane staining pattern

CD10 antibody is positive in 75% of acute lymphoblastic lymphomas/leukemias originating from pre-B cells and 63% of pre-T cells ⁽⁷⁾. This reagent also identifies a variable number of chronic myeloid leukemias in blastic phase ⁽²³⁾, Burkitt lymphoma ⁽²⁴⁾, non-Hodgkin lymphomas of the follicular center (70% a variable number of diffuse large follicular B-cell lymphomas ⁽²⁵⁾). Among the latter, CD10 specifically identifies those of germinal phenotype that are concomitantly CD10 and Bcl-6 positive with MUM1 negativity ⁽²⁶⁾. CD10 also marks 10% of hairy cell leukemias ⁽²⁷⁾. Additionally, the follicular helper T cells that characterize angioimmunoblastic T lymphoma are also CD10-positive ⁽²⁸⁾, allowing their distinction from other T lymphomas ⁽²⁹⁾. CD10 is also positive in some follicular mantle lymphomas, multiple myeloma precursor B cells ⁽³¹⁾ and 32% of primary thymic B lymphomas ⁽³²⁾.

In contrast, CD10 is negative in small B cell lymphoma, marginal zone lymphoma and lymphoplasmacytic lymphoma. In the case of T cells, CD10 has been demonstrated in most cases of angioimmunoblastic T cell lymphoma while peripheral T cell lymphomas are negative.

6.1.2 CD10 in neoplasms of other organs

Two different staining patterns are possible:

- A) Only from the apical cell surface, in well-differentiated tumors of the colon, pancreas or prostate.
- B) With mixed pattern (membranous and cytoplasmic with or without Golgi apparatus reinforcement) in other carcinomas, less differentiated carcinomas and other neoplasms of sarcomatoid or melanocytic origin.

6.1.2.1 Colon:

CD10 expression is increased during carcinogenesis of this tumor and allows identification of CD10+ stromal fibroblasts adjacent to areas of high-grade dysplasia ⁽³³⁾. Likewise, the presence of this marker correlates with the tumor aggressiveness and rate of subsequent liver metastases ⁽³⁴⁾.

6.1.2.2 Urinary bladder:

CD10 is present in 40% of carcinomas of this origin, especially in high-grade ones, and correlates with their cytological grading and clinical stage ⁽³⁵⁾.

6.1.2.3 Breast:

CD10 is a good marker to identify myoepithelial cells in mammary carcinoma in situ ⁽³⁶⁾, although sometimes this antibody also stains epithelial (apocrine and metaplastic) and benign and malignant mesenchymal neoplasms of this location. However, it is negative in most ductal and lobular carcinomas of this location ^(37, 38).

6.1.2.4 Kidney:

CD10 is expressed in 90-95% of clear cell carcinomas, papillary carcinomas and Xp11 translocation carriers, which allows its distinction from the eosinophilic variant of chromophobe carcinoma and oncocytoma ⁽³⁹⁾. In the rest of the renal neoplasms, its expression is highly variable, which limits its diagnostic usefulness ⁽⁴⁰⁾.

6.1.2.5 Pancreas:

CD10 is useful for differentiating between mucinous cystic neoplasms (CD10+) from their intraductal analogs (CD10-) and identifying solid and papillary neoplasms of this location ⁽⁴¹⁾.

6.1.2.6 Gynecopathology:

CD10 is useful for differentiating endometrial stromal tumors from benign and malignant smooth muscle neoplasms of the uterine wall, which are CD10 negative ⁽⁴²⁾, as well as for identifying endometriosis and mesonephric hyperplasias by differentiating them from other types of endometrial or ovarian carcinoma ⁽⁴³⁾. Similarly, CD10 distinguishes primary endometrial or ovarian clear cell carcinoma, which is CD10 negative, from CD10+ renal metastatic carcinoma ⁽⁴⁴⁾ and endometrial stromal sarcoma from uterine smooth muscle tumors ⁽⁴²⁾.

6.1.2.7 Liver:

In hepatocarcinomas, staining with CD10 allows the identification of its canalicular pattern ⁽¹²⁾, although for the immunohistochemical diagnosis of these neoplasms it is recommended to use CD10 together with other markers of hepatocyte lineage.

6.1.2.8 Skin:

The CD10 antibody is useful for the diagnosis of different cutaneous adnexal neoplasms ⁽⁴⁵⁾, with the main application to differentiate between basal cell carcinoma and trichoblastoma ⁽⁴⁶⁾, as well as to identify atypical fibroxanthoma and distinguish it from sarcomatoid squamous cell carcinoma. It has also been described in some forms of malignant melanoma, which is associated with aggressive tumor behavior ⁽⁴⁷⁾.

6.1.3 Other applications

CD10 positivity contributes to the diagnosis of many other neoplasms ⁽⁴⁸⁾, including germ cell neoplasms, choriocarcinoma, some vascular tumors such as epithelioid hemangioendothelioma, some gliomas, melanoma, prostatic and pancreatic adenocarcinoma, rhabdomyosarcoma and other sarcomas, including Ewing's sarcoma, schwannoma and mixed Müllerian tumors. A correlation has also been observed between CD10 expression levels in cancer and a higher histological grade, tumor size, vascular invasion and survival rate in patients with various solid tumors ⁽⁴⁸⁾.

6.1.4 Possible indications of prognostic and predictive use

There are many published observations where neoexpression or overexpression of the CD10 marker acts as a predictor of the clinical behavior of the tumor or is associated with lower rates of neoplasm free time or patient survival ⁽³⁾, mainly in breast cancer ^(49, 50), where, additionally, CD10 expression by stromal fibroblasts is associated with chemoresistance and poor prognosis ⁽¹⁹⁾; stromal lung ⁽⁵¹⁾; head and neck cancer ⁽⁵²⁾ and malignant melanoma ⁽⁵³⁾. In hematopoietic tumors, CD10 expression is classically identified as a good prognostic marker in diffuse large B-cell lymphomas ⁽⁵⁴⁾.

6.2 Clinical specificity and sensitivity

In the pursuit of clinical validation, we deemed it appropriate to rely on a comparative analysis of the positive cases detected using our product against those documented in the literature for similar products. This approach is grounded in the following considerations:

The CD10 (56C6) enjoys a well-established reputation as a diagnostic marker, bolstered by robust support in the existing literature.

The CD10 (56C6) serves as a diagnostic tool that primarily leans on qualitative assessment rather than quantitative measures. It is largely contingent on the clinical judgment of pathologists, who, in conjunction with other histopathological data and additional markers, arrive at a diagnosis.

The CD10 product is a monoclonal antibody with broad diagnostic potential but low specificity, so its immunohistochemical interpretation is always performed in the morphologic context of the lesion analyzed and in conjunction with other immunohistochemical markers. This way, the CD10 antibody has proven its utility in the diagnosis and classification of lymphomas, renal-cell carcinomas or even sarcomas, among others.

In relation to the use of the CD10 antibody as a diagnostic support and considering that its relatively low specificity is widely recognized in the literature (it can be positive in very numerous histological types of benign and malignant neoplasms), with limited sensitivity (there are only three neoplasms with sensitivity above 90%), to evaluate the metrological traceability of the antibody we show the comparative results between the literature and the CD10 product from VITRO S. A in the three tumors where the CD10 antibody shows sensitivity higher than 85% as well as remarkable specificity and intense staining in the presence of a concordant histology:

1. Clear cell renal cell carcinoma
2. Endometrial stromal sarcoma
3. Atypical fibroxanthoma of the skin

The sensitivity of VITRO, S.A. data has been compared to the sensitivity value found in the literature.

Table 2. Comparison of VITRO, S.A.'s sensitivity value with data obtained in literature.

CD10	Sensitivity Literature	Specificity Literature	Sensitivity clinical series of VITRO, S.A
Clear-cell renal-cell carcinoma (11)	90%	Very low, does not discriminate RCC from adrenal tumors and other neoplasms.	86% (43 cases out of 50)
Endometrial stromal sarcoma (42)	100%	High (89%) in the proper histological context <5% of leiomyomas and 0% of positive leiomyosarcomas	100% (6 cases out of 6)
Atypical fibroxanthoma (AFT) (47)	87.1%	High (87%) in spindle cell tumors of the skin within the appropriate clinical context (87% AFT positive with 0% desmoplastic melanomas and only 13.6% desmoplastic squamous cell carcinomas).	90% (9 cases out of 10)

To evaluate tumors where the antibody has shown low sensitivity and weak membrane staining, cases with focal and/or weak positivity have been selected among the negative cases of the previous series and some other positive tumors, essentially follicular lymphomas and moderately differentiated hepatocarcinomas among those shown in the table.

Test results should be evaluated in conjunction with other diagnostic technologies, patient's medical history and other findings.

Since the performance characteristics may vary with pre analytical conditions, sample handling and storage, reagents, it is recommended that each laboratory establish its own verification of the results.

7 PRODUCT DESCRIPTION

7.1 Composition

Ready-to-use mouse anti-CD10 monoclonal antibody supplied in liquid format in an pH7.6 buffer containing the stabilizing protein. Although it is not a sterile product, microbiological contamination is controlled, although not monitored since the solution contains sodium azide as a bacteriostatic and bactericidal agent. This additive improves device performance without affecting measurement results. The amount of active antibody in the sample is 0.167-0.333 µg/ml.

The antibody is also available in concentrated format supplied in liquid form in a pH7.6 buffer containing stabilizing protein with added sodium azide as a bacteriostatic and bactericidal agent.

7.2 Clone

56C6 (identified in the batch field of the product label)

7.3 Immunostaining pattern (samples and controls)

Cell membrane

7.4 Immunogen

Recombinant fusion protein corresponding to the external domain of the CD10 molecule.

7.5 Immunoglobulin source and isotype

Mouse IgG1

7.6 Intended Use

Professional laboratory use. In vitro diagnosis in humans.

7.7 Application

Immunohistochemical determinations on paraffin-embedded tissue.

7.8 Storage conditions

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

7.9 Validity period

The product, if preserved in the established storage conditions, can be used up to the expiration date indicated on the label. 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 product warranty is no longer valid.

The reagent, once opened and in use, is stable up to 19 months and at most up to the expiration date indicated on the label if stored and used according to the manufacturer's recommendations.

7.10 Special instructions for manipulation

This reagent is specially designed for its use in the immunostainer VitroStainer 42, although the use of other automated immunostainers and manual procedures is possible as long as the reagent is validated locally according to the conditions existing in each laboratory.

For a concentrated format, the recommended dilution is 1:50 (it has been validated with the immunodetection system Master Polymer Plus Detection System-Peroxidase ref. MAD-000237QK). The recommended buffer for this product is MAD-004048R Antibody diluent from VITRO, S.A.

8 CALIBRATORS AND CONTROL MATERIALS RECOMMENDED FOR LABORATORY USE DURING IHC TEST

8.1 Internal positive control

Any of the normal cells described in section 5 when present in the test sample.

8.2 External positive control

Tissue section from normal tonsil/spleen with previous expression of this marker, ideally placed on the same slide as the test specimen.

8.3 Negative control

Preparation homologous to the test sample incubated with a non-specific isotype antibody for CD10.

8.4 External quality controls

It is highly recommended that each laboratory establishes an external quality assurance control system for its immunohistochemical procedures through recognized intercomparison programs.

9 SAMPLE TYPES

Paraffin-embedded tissue sections 2 to 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 14 Warnings and Precautions). 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 CD10 molecule expression levels by immunohistochemistry techniques on formalin-fixed and paraffin-embedded tissue.

10 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 deparaffining of the sections, heat-induced antigen retrieval and subsequent washes in buffer. It is highly recommended that the samples used for immunostaining have been freshly sectioned on the microtome (freshly cut or cut the day before the test is performed).

In order to know the technical conditions of this antibody on VitroStainer 42, contact your authorized Supplier/Distributor.

Although this antibody clone has been successfully used in antigen retrieval systems and other immunostainers than those mentioned above, its use in them requires prior validation.

11 ANALYTICAL PRINCIPLE OF THE METHOD AND PERFORMANCE CHARACTERISTICS

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.

11.1 Analytical sensitivity and specificity

This antibody is specific for the human CD10 and does not cross-react with other molecules. Likewise, the concentration at which it is supplied, determined by dilution of the culture supernatant, is suitable for immunohistochemical determination by kits using peroxidase or alkaline phosphatase polymers.

11.1.1 Analytical sensitivity

Analytical sensitivity was carried out with both manual and automated procedures on VitroStainer 42 instrument, to confirm the optimal dilution and time of incubation of the CD10 Antibody assures the good results under the final selected conditions. Results showed that optimal results on target cells without nonspecific labeling or background noise were obtained.

11.1.2 Analytical Specificity (Interference / Cross-reactivity)

Analytical specificity testing was performed in order to find interference substances or cross reactivity results.

- **Interference analysis:** Analysis substances used during the process which demonstrated a loss of staining activity and thus should not be used in conjunction with the processing of samples to be stained using CD10 Antibody. The only known interference comes from alcohol containing fixatives, which demonstrated a loss of staining activity and thus should not be used in conjunction with the processing of samples to be stained using this product (59)
- **Cross reactivity:** The results obtained under the optimum conditions defined for the test are as follows:

Table 3. Cross reactivity results. Positive controls containing internal negative controls

Tissue	Positive	Negative
Tonsil/spleen	Germinal centre B lymphocytes, isolated	Marginal and mantle zone interfollicular B lymphocytes, T lymphocytes; plasmocytes.
	myofibroblasts and cells of the	Lining squamous epithelium and of the secretory cells of the salivary acini.
	salivary acini	Vascular endothelium
Normal breast (ductal epithelium)	Myoepithelial cells	Luminal/secretory cells
Result	Optimal	Optimal

All the results were analyzed by a pathologist.

Final optimal results were accorded with the acceptance criteria.

11.2 Precision

The determination of CD10 by this monoclonal antibody is reproducible if the developing kits recommended in the instructions for use are used.

11.2.1 Repeatability

The repeatability of the method was analyzed using positive normal tonsil controls previously tested with positive results. The tests were performed by the same operator, at a single location and with the same instrument, and using the same batches of reagents on three consecutive days. All results were analyzed by a pathologist. It is concluded that the technique is 100% repeatable on all 3 days.

11.2.2 Reproducibility

The reproducibility of the method was analyzed using the automated method and optimized conditions for this marker by processing the same positive normal tonsil controls but performing the tests with different lots of reagents, operators and equipment. It is concluded that the technique is 100% reproducible.

11.3 Limits of detection

As there are currently used no routinely applicable quantitative procedures to ensure limits of detection on these qualitative non-linear products, as an alternative to these methods and to ensure dilution of the antibody, the following three internal procedures have been used:

1. Progressive dilution of the antibody in VitroStainer 42, on control tissues, confirming for experience the dilution located in the median between excessive staining not acceptable for diagnosis and weakly positive, which does not ensure the exclusion of false negatives.
2. External validation of the selected dilution in several clinical series of reference laboratories and in the NordiQC Quality Control and Quality Assurance Centers in immunohistochemistry of the Spanish Society of Anatomic Pathology (SEAP).
3. Results: The NordiQC Quality Control and Quality Assurance Centers in immunohistochemistry of the Spanish Society of Anatomic Pathology Reports can be requested to VITRO.
4. Review of the dilution used in the scientific literature for this same type of clone and/or antibody evaluated.

11.4 Measurement range

Not Applicable. The immunohistochemical process is not a quantitative method, it is a qualitative one where the marking obtained is interpreted as positive or negative complemented with the corresponding morphological and histological studies of each lesion and the appropriate controls.

12 PRODUCT LIMITATIONS

1. Not recommended for Western-blotting and flow cytometry techniques.
2. The use on frozen tissue has not been evaluated.
3. This antibody is intended for in vitro diagnostic (IVD) use by qualified laboratories only.
4. Test results should be evaluated in conjunction with other diagnostic technologies, patient's medical history and other findings.
5. Since the performance characteristics may vary with pre analytical conditions, sample handling and storage, reagents, it is recommended that each laboratory establish its own verification of the results.
6. Each laboratory must define its own use ratings for the product following the manufacturer's instructions and calibrate the equipment where they are to be used.
7. Due to inevitable variability in immunohistochemical procedures and variables, appropriate positive and negative controls must be used and documented, and the results are to be interpreted by a qualified pathologist.
8. Prediluted antibodies, which are provided in a ready- to-use optimally diluted format, must be used explicitly as instructed. Improper handling and processing of tissue samples and reagents, and any deviation from the recommended procedures outlined herein, may compromise the validity and/or analysis of the results.

9. If concentrated antibodies are provided in a format that requires dilution in the optimized buffer, in the context of appropriate validation by the user, any diluent different than that specified in the package insert must also be validated by the user to ensure proper compatibility with the antibody. Once diluted, any deviation from the recommended procedures outlined herein may compromise the validity and/or analysis of the results.

10. The potential for unexpected results in patient tissue specimens cannot be discarded due to inherent biological variability in the expression of certain antigens.

11. The potential for false positive results in patient tissue specimens cannot be discarded due to the possibility of non-immunological binding of substrate reaction products or proteins. False positive results may also occur subject to the type of immunostaining technique used, or due to the activity of pseudoperoxidase, endogenous peroxidase, or endogenous biotin.

12. Due to the effect of autoantibodies or natural antibodies, normal sera from an animal source the same as the secondary antisera may result in false negative or false positive results when used in blocking steps.

13 EQUIPMENT AND PRODUCTS REQUIRED

13.1 Supplied with the reagent

Besides the antibody the product does not include any additional reagents needed.

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:

- VitroStainer 42
- Pretreatment Kit
- Detection system
- 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 to optimize the process, it is recommended to use the different amplification, development and counterstaining products in form of kits manufactured by VITRO, S.A., in the immunostainer, as well as the specific buffers and consumables for these instruments.

This antibody has been optimized and validated with the detection system commercialized by VITRO, S.A. (MAD-000237QK, QP, QKVS) in the automated VitroStainer 42 although the product can work well with any other polymer-based detection system and with other automated systems or manual immunostaining

procedures. If the product is to be used with detection systems and 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 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:**
 - For its handling in research, the presentation as a concentrated antibody is more suitable, which allows investigating the most appropriate antibody dilution and conditions of use in each case.
 - The reagent only works properly with a reagent chain suitable for amplification and development, so its use with other systems than the recommended one should be subject to close review and/or eventual validation in each laboratory.
 - 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 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.
- **Summary of Safety and Performance (SSP):** The Summary of Safety and Performance (SSP) of this product will be available on EUDAMED database (subject to EUDAMED availability). This summary is also available on request by e-mail at regulatory@vitro.bio.

• **Recommendations for the Safe Handling of Samples Suspected of Containing Prions:**

The following protective measures are recommended for professional personnel handling immunohistochemistry (IHC) samples suspected of containing prions:

- Personal protective equipment (PPE): full PPE should be worn, including a mask (preferably FFP2 or higher), protective goggles or face shield, head cover, and shoe covers.
- Precautions during microtome sectioning: use disposable blades and avoid the use of aerosols or compressed air to clean the microtome area.
- Surface and equipment decontamination: disinfect with sodium hydroxide (NaOH) 1N or sodium hypochlorite at 2%.
- Waste management: all disposable materials must be discarded as special biological risk waste, in accordance with the applicable national regulations on hazardous healthcare waste.

- **Waste disposal:** The handling of waste 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 waste 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:

Table 4. Classification of waste generated by this kit according to the European Legislation. *ELW: European Legislation of Waste

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 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.	160506	"Laboratory chemicals consisting of or containing dangerous substances, including mixtures of laboratory chemicals"

***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:

	In vitro diagnostic medical device		Expiration date
	Catalog number		Temperature limit
	Lot code		Manufacturer
	Refer to the instructions for use		Sufficient content for <n> assays
	Safety data sheet		Distributor
	Importer		

17 CHANGE LOG

Date	Description
08-07-2022	Creation of the document.
03-11-2022	Update of the document to the new template. (Table included in Section “13. Warnings and precautions” were modified)
28-01-2023	Update of the document to the new template. (Section “6. Description of the product” and “7. Analytical performance characteristics” were modified)
25-05-2023	Updated sections 2, 6.1, 6.8, 7.3.1, 7.3.2, 7.4
28-06-2023	Updated section 2 intended purpose of the product
25-08-2023	Section 13 “Warnings and Precautions” is revised to update the information about “Summary of Safety and Performance (SSP)”
17-02-2025	- Section 3 “Other possible application of the product” is added - Sections 1, 7.10, 10, 11.1.1, 11.3, 13.2 are updated to include VitroStainer 42 instrument - Section 16 is updated to include Importer and Distributor symbols
03-03-2025	The document is updated to the new version of the template.
08-09-2025	Section 7.9 is updated to include conditions in use of the product. Section 7.10 is updated to include the recommended buffer for the concentrated format. Section 9 is updated to include correct reference to Section 14 Section 14 is updated to include “Recommendations for the Safe Handling of Samples Suspected of Containing Prions”