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Immunoglobulin Lambda Light Chains CISH Kit (Using Zytovision technology)

DESCRIPTION:

This kit contains reagents to perform manual or automated chromogenic in situ hybridization (CISH) technique on human tissue sections fixed in buffered formalin and embedded in paraffin.
This kit does not contain a visualization system and is sufficient for the realization of 5 or 20 determinations following the recommended protocol. The best results have been obtained using the visualization system based on the Master Polymer Plus available in the Vitro S.A catalogue.

Presentation: The general reference / presentation for this kit is:

MAD-001894QH-MP	**5 tests**
MAD-001894QH	**20 tests**

This reference is for presentation packaging in Low Density Polyethylene (LDPE) dropper. In case the user wants other presentations (references / different volumes) must contact the supplier.

Intended Use IVD: 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in this document, the user must previously check its correct functionality considering that the product's warranty is no longer valid.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 Autostainer or for manual staining.
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 immunohistochemical 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.

SPECIFICITY

The immunoglobulin molecules (Igs) are composed of two pairs of polypeptide chains, two heavy (IgH of 50-70 kD) and two light chains (IgL 23 kD), each of the light chains containing two successive domains: the first constant domain and the second a variable domain. Light chains are covalently linked to each correspondent

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heavy chain and are assembled through somatic rearrangement of the hypervariable region gene segment V (D) J. This process occurs during the early stages of the maturation of B lymphocytes, so that first (in the pro-B stage) the IgH chain rearrangement occurs to define the type of immunoglobulin that each cell will secrete and subsequently (in the pre -B stage) the IgL chain rearrangement occurs.

In humans there are two IgL isotypes: Kappa (κ), encoded by a gene located on second chromosome and the Lambda (λ) with the coding gene located on chromosome 22. Each of the B-lymphocytes produces heavy chain immunoglobulins (IgG, IgM, IgA, IgD or IgE) and light chain immunoglobulins κ (IgLκ) or λ (IgLλ) but never both simultaneously. Under normal conditions, 60% of B lymphocytes produce κ light chain immunoglobulinas with the rest represented by the λ chain. Therefore in all reactive lymphoid proliferation a mixed population of κ and λ chains positive cells is recognized, which is granted as a polyclonal expression.

As all malignant neoplasias, B cell lymphomas are clonal tumors originating from a transformed cell; hence, they are capable of producing only one type of immunoglobulins light chains, which is known as a monoclonal expression. Therefore the determination of the relationship between the two light chains of immunoglobulins in a cell population represents an essential tool for establishing the clonality in B cell lymphomas and neoplastic or reactive plasma cell dyscrasias.

This probe is designed for the detection of mRNA of the lambda light chains (λ) through chromogenic in situ hybridization (CISH) techniques in tissues or cells fixed in 10% buffered formalin and paraffin embedded.

DIAGNOSTIC APPLICATIONS:

Determining the relative ratio of the two existing light chains of immunoglobulins by the chromogenic in situ hybridization techniques is suitable for the detection of B cell lymphoproliferative disorders clonality, providing substantial support for a lymphoma diagnosis when compared with any lymphoid hyperplasia or other reactive lymphadenitis. In the same line it has a diagnostic value in plasma cell dyscrasias and leukemia, myeloma and plasmacytoma.

PATTERN AND CONTROLS

Pattern: Cytoplasm
Positive Control: Tissue section from tonsil.
Negative Control: Homologous preparation to the sample to be tested incubated with a nonspecific mRNA for Lambda light chains.

LIMITATIONS OF REACTIVE
The use of frozen tissue has not been evaluated.

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 ANALYTICAL METHOD (in situ hybridization)

In situ hybridization (ISH) allows the detection of specific sequences of DNA or RNA in histological and cytological samples without losing morphological details. The ISH technique is based on hybridization between a DNA or RNA sequence specifically labelled (probe) and a sequence of DNA or RNA present in the sample. If there is any complementarity between the sequences the hybridization occurs, which results in a hybrid product. Hybrids can be easily viewed by a chromogenic immunohistochemical staining procedure (CISH) directed toward specific marker of the probe employed.

The ISH technique is highly sensitive, specific and easy to perform, and in addition, contains no radioactive products. The reagents supplied in this kit are perfectly matched and therefore, the kit is an easy to use tool to perform CISH.

COMPONENTS AND REAGENTS INCLUDED IN THE KIT:

MAD-001894QH-MP			
Proteinase K Concentrated Solution (10 mg/mL)	MAD-001892QE-CM	5 tests	50 µl conc.
Digoxigenin-Labeled Human Ig-Lambda Probe	MAD-001894HS-MP	5 tests	50 µl
Digoxigenin (HY-A.1)	MAD-001892QD-4	5 tests	4 ml pred.
MAD-001894QH			
Proteinase K Concentrated Solution (10 mg/mL)	MAD-001892QE	20 tests	200µl conc.
Digoxigenin-Labeled Human Ig-Lambda Probe	MAD-001894HS	20 tests	200µl
Digoxigenin (HY-A.1)	MAD-001892QD-4	20 tests	4 ml pred.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

- Visualization system recommended - **Master Polymer Plus Detection kit** - **MAD-000237QK**
- Humid incubation chamber
- Slides treated with silane or electrically charged - **IHC Treated Slides - 100 Units - MAD-15-188-55/100**
- Coverslips
- Thermal plate or oven (37 ° C)
- Dewaxing and hydratation battery (xylene, absolute ethanol and of 80% and 70% concentrations)
- TBS buffer - **Tween 20 - TBS Tween 20 Buffer 10X q.s. 1L - MAD-004077R**
- Micropipettes
- Contrast hematoxylin - **Haematoxylin - MAD-108.1000**
- Optical microscope

TECHNICAL PROTOCOL FOR CISH LABDA LIGHT CHAIN STAINING USING MASTER DIAGNOSTIC DETECTION KIT

This protocol is preferred for conducting CISH on tissue sections fixed in buffered formalin and embedded in paraffin.

1. Dewax and rehydrate

a. Incubate slides with paraffin tissue sections in the oven at 60 ° C overnight.
b. Dewax:
- Xylene 10 min 2x

c. Rehydrate
- Absolute ethanol 2x 5 min
- Ethanol 80% 5 min
- Ethanol 70% 5 min
- Distilled water (10 min)

2. Enzymatic digestion

a. Dilute 5 µl of **Proteinase K Concentrated Solution** in 2 ml of **TBS buffer**.
b. Apply the resulting solution onto the tissue and incubate 8 min at room temperature (RT).
c. Wash 3 times with TBS buffer at RT

3. Hybridization

a. Apply 10 µl of **Digoxigenin-Labeled Human Ig-Lambda Probe** on the tissue.
b. Place a coverslip on the section.
c. Place the slide into a wet camera and incubate 40min at 62°C.

4. Detection and visualisation (manual or automatic)

a. Remove the coverslip and wash 3 times in TBS at RT
b. Apply 200 µl of **Peroxidase Blocking Reagent** on the tissue and incubate 10 min at RT
c. Wash 3 times in TBS.
d. Apply 200 µl of **Digoxigenin (HY-A.1)** antibody on tissue and incubate for 10 min at RT.
e. Wash 3 times in TBS.
f. Apply 200 µl of **Primary Antibodies Amplifier Master** on the tissue and incubate 15 min at RT.
g. Wash 3 times in TBS.
h. Apply 200 µl of **Master Polymer Plus HRP** on the tissue and incubate 20 min at RT.
i. Wash 3 times in distilled water
j. Mix a drop of **DAB Chromogen Concentrate** with 1 ml of **DAB Substrate Buffer** solution and applying the resulting solution onto the tissue; incubate for 7 min at RT
k. Wash 3 times in distilled water.

5. Contrast staining and mounting

a. Stain with **contrast haematoxylin**.
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.

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 using its contact information as mentioned above.

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 is not responsible for damage, personal injury or economic loss that this reagent can be involved.

REFERENCES:

1. Ståhlberg A., Åman P., Ridell B., Mostad P., Kubista M. Quantitative Real-Time PCR Method for Detection of B-Lymphocyte Monoclonality by Comparison of κand λImmunoglobulin Light Chain Expression Clin Chem. 2003 Jan;49(1):51-9.
2. Levy R, Warnke R, Dorfman RF, Haimovich J. The monoclonality of human B-cell lymphomas. J Exp Med 1977;154:1014-1028.
3. Marshall-Taylor CE, Cartun RW, Mandich D, DiGiuseppe JA.Immunohistochemical detection of immunoglobulin light chain expression in B-cell non-Hodgkin lymphomas using formalin-fixed, paraffin-embedded tissues and a heat-induced epitope retrieval technique. Appl Immunohistochem Mol Morphol. 2002;10:258-262.
4. Leers MP, Theunissen PH, Ramaekers FC, Schutte B, Nap M.Clonality assessment of lymphoproliferative disorders by multiparameter flow cytometry of paraffin-embedded tissue: an additional diagnostic tool in surgical pathology. Hum Pathol. 2000;31:422-7.

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		

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