EXPRESS ISH®

Express In-Situ Hybridization

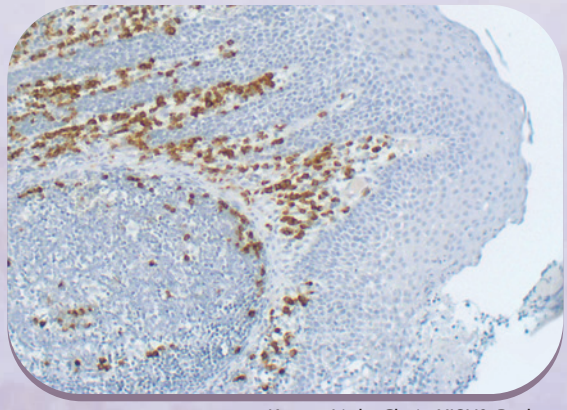
The Best Way to Bring Molecular Diagnostics into your lab!

American MasterTech
scientific laboratory supplies

With any laboratory oven and these items shown below,

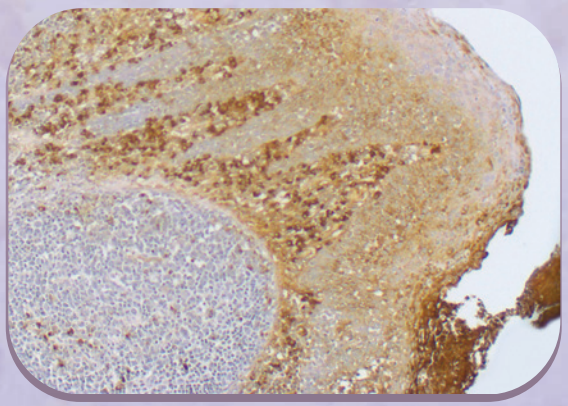
StainTay, 30% Hydrogen Peroxide, Proteinase K, PBS 7.4, Steam Distilled Water, XISH® Probe, Anti-Digoxin, DAB, Polymer HRP, and Hematoxylin.

you can be producing excellent molecular diagnostic results that looks like this....



Kappa-Light Chain XISH® Probe

instead of this....



Kappa-Light Chain IHC Antibody

No expensive equipment, and no toxic chemicals like formamide are required!

What is XISH® ?

XISH® is a molecular diagnostic system that uses single stranded DNA probes to target messenger RNA for gene expression. It's ***faster, far more specific, and far more*** reliable, than any other CISH or FISH technology in the market today.

XISH® is so easy to Perform! It's very similar to IHC!

1. First off, we apply Proteinase K to expose the target instead of the antigen retrieval that you normally perform in IHC.
2. Then, we apply the probe and incubate it in a humidity chamber for just one hour at 62°C!
3. After that, we apply a primary antibody, secondary antibody, DAB, counterstain with Hematoxylin, and coverslip like normal!

That's it! Best of all, anti-digoxin and an anti-mouse polymer HRP are the only primary and secondary antibodies used with all of our probes!

Why use XISH® instead of FISH, CISH, or IHC?

FISH or CISH:

Can Require Expensive Equipment, Toxic Formamide for Denaturation steps, and Multiple Rinse Buffers. It can take as long as 48 hours to hybridize the probe and the result can have weak signals due to the short length of the probes that are currently in the market. With FISH, Fluorescent dyes fade and cannot be archived.

IHC

Did you see the picture on the front page? Need we say more!? IHC requires signal interpretation due to the background staining of the highly unspecific proteins that are targeted by this technology.

T: 1-800-860-4073
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HISTOSONDAS OF THE IMMUNOGLOBULIN LIGHT CHAINS (KAPPA OR LAMBDA)

Assay of 4 vials (Kappa Light Chain or Lambda Light Chain) for 20 individual reactions. 65µl for reactions

Product classification:
For Research Use only (RUO)

INTRODUCTION

Immunologically competent B lymphocytes develop from a lymphoid stem cell until acquiring a receptor consisting of a single immunoglobulin molecule specific for a determined antigen. These immunoglobulin molecules are formed by two identical heavy chains and by two identical light chains (kappa or lambda).
In humans, in normal or reactive lymphoid tissues, approximately 60% of the B lymphocytes bear kappa light chains and the remaining 40%, lambda light chains. In B lymphocyte tumors, there is generally only one clone that proliferates, be it kappa or lambda, in a monoclonal way.
In humans the gene that codes for kappa light chains is located on chromosome 2, and the gene that codes for lambda light chains is located on chromosome 22. The variable regions for genetic recombination are located in the first 5' half of these genes, while the constant regions are located in the 3' half. There is only one functional gene for the constant region of k light region, and four functional genes for the constant region of λ light chains (Cλ1, Cλ2, Cλ3, and Cλ7).
Detection of monoclonality is one of the most important tools for differentiating B lymphoid tumors from reactive processes. In situ hybridization technique offers an important advantage over immunohistochemistry, as it virtually lacks background, and allows a clean and sharp viewing of the histological preparation. It is also useful to differentiate cells that have absorbed immunoglobulins, and are therefore detectable by immunohistochemistry, but in fact do not produce immunoglobulin, as occurs with the Reed-Sternberg cells of Hodgkin's disease.

INTENDED USE

For Research Use only (RUO)
The Histosondas of the Immunoglobulin Light Chains are useful for the study of monoclonality in lymphoid tumors, lymphoproliferative syndromes, myelomas and for the study of immunodeficiency associated lymphoproliferative syndromes.

WARNINGS AND PRECAUTIONS

The Histosondas of the Immunoglobulin Light Chains have been designed for their professional use in research and must be manipulated by qualified and accordingly trained personnel.
In order to obtain the best results, the instructions contained in the manual must be followed. Any change to the indicated temperatures, times or any other step of the process can lead to poor results.
This product contains sodium azide as a preservative (NaN_3). Sodium azide is below the threshold level of 0,1% (w/v) of the total, and therefore it is not considered to be a hazardous substance. Small quantities may be eliminated in the waste water system.

COMPONENTS

4 tubes with the necessary amount of Histosonda Kappa or Lambda (as indicated on the label) for developing 20 single reactions.
The Histosondas of the Immunoglobulin Light Chains consist of a fragment of single-stranded DNA with a length of between 153 and 182 nucleotides, complementary to expressed RNA. These probes have been labeled with Digoxigenin.

STORAGE CONDITIONS

Supplied reagents are stored at 3-7ºC until expiration date. Do not use after expiration date.

SAMPLES

Block sections of formalin fixed paraffin embedded tissues. Sections of 4-6 micrometers in width are sufficient to conduct the study.

INTERPRETATION OF RESULTS

Samples in which immunoglobulin light chains expression is observed will show a brownish color in the cell cytoplasm, which will contrast over the blue-violet background given by hematoxylin staining. The pathologist will evaluate the results according to their experience, drawing conclusions from the staining of the sample, in parallel with the staining observed in the positive and negative controls for expression of this gene.

ASSAY LIMITATIONS

The Histosondas of the Immunoglobulin Light Chains have been optimized to detect RNA expression in formalin-fixed, paraffin-embedded tissues. Its use is not recommended for other types of samples or preparation techniques.
The correct operation of this product has been validated using the protocols indicated in the instructions manual. The use of other procedures or the modification of the recommended protocols may lead to erroneous results.
The results from this assay must be evaluated by the pathologist in combination with the rest of available patient clinical data.
In order to obtain optimal and reproducible results it is important to rigorously maintain the time and temperature conditions indicated in the procedure.

BIBLIOGRAPHY

1. Peter J. Delves And Ivan M. Roitt: Immunoglobulin genes. In ENCYCLOPEDIA of IMMUNOLOGY. Page 1323. Second Edition, ACADEMIC PRESS LIMITED (1998)
2. Weiss LM, Movahed LA, Chen YY, Shin SS, Stroup RM, Bui N, Estress P, Bindl JM. Detection of immunoglobulin light-chain mRNA in lymphoid tissues using a practical in situ hybridization method. Am J Pathol (1990) Oct; 137(4):979-88.
3. Ruprai AK, Pringle JH, Angel CA, Kind CN, Lauder I. Localization of immunoglobulin light chain mRNA expression in Hodgkin's disease by in situ hybridization. J Pathol.1991 May; 164(1): 37-40

PROCEDURE

For Digoxigenin- labeled probes

BASIS OF THE METHOD

Chromogenic In Situ Hybridization (CISH) is a technique used to determine the presence of a DNA or RNA sequence or to study gene expression, as well as for the simultaneous evaluation of tissue morphology by white light microscopy. A labeled probe of known sequence hybridizes with its target in the tissue being studied. This hybridization is then detected by an immunohistochemical process.
The Histosondas targets are messenger RNA and they are therefore designed to detect and visualise gene expression.

HistoSonda Protocol

Needed accessories for this protocol and not provided with the product
Proteinase K, Anti-Digoxin Antibody, polímero comercial anti-mouse HRP comercial polymer, Diaminobenzidine (DAB) solution.

Before starting pre-heat the humid chamber to 62ºC.

1a. Deparaffinization

1. Heat slides at 62°C for 10mins using a hot plate or incubator.
2. Immerse slides in:
   a. Xylene: 10 mins
   b. Xylene: 5mins
   c. Absolute alcohol (ethanol or isopropanol): 1min X 2
   d. Alcohol 96%: 1min X 3
   e. Methanol containing 0.3% H_2O_2 or 3% H_2O_2 only: 5mins
3. Wash well with distilled water, leave standing in water.

1b. Inhibition of unspecific DNA binding (optional)

*Generally this step is not necessary unless the tissue to be hybridized contains a great quantity of polymorphonuclear eosinophil leukocytes (generally **bone marrow** and **gastric tissues**).*

1. Place slides in boiling distilled water for **30 seconds** and immediately transfer to distilled water at room temp.

! Important: excess heat treatment will result in background staining. Remove slides immediately. **!**

2. Deproteinization

1. Prepare a dilution of any commercial Proteinase K at a work concentration of 0,033 mg/ml in PBS pH 7.4.
2. Remove excess water from individual slides with tissue paper.
3. Cover tissue sections with the 200µl of Proteinase K and incubate in a humid chamber for exactly 10mins at room temp.
4. Wash well with distilled water.
5. Transfer to PBS pH 7.4 for 2mins.

! (**Important:** if the slides have been boiled in the microwave previously only use Proteinase K for 5 minutes as the heat treatment will have significantly sensitized the tissue sections. **Bone marrows require a Proteinase K digestion of 20min at 55ºC after heat treatment**) **!**

3. Incubation with the probe

1. Remove excess buffer from sections as described previously.
2. Add 65µl of probe to the tissue section ensuring the entire section is completely covered and avoiding air bubbles.
3. Place the slides in a horizontal position in a humid chamber and close well.
4. Incubate at 62ºC for 1hr.

4. Washing the probe

! *The probe solution is very viscous. It is **very important** to wash the slides well to ensure the probe solution is completely removed.***!**

1. Wash the surface of the section vigorously with PBS pH 7.4 to remove the probe.
2. Agitate in PBS for 5mins.

5. Revealing the probe

Protocol for manual revealing of the probe.

1. Remove excess buffer from sections as before.
2. Cover sections with 100µl of Anti-Digoxin (not provided with this product) following manufacturer's instructions and incubate in a humid chamber for 30mins at room temp.
3. Wash vigorously with PBS pH 7.4 agitate in PBS for 1min.
4. Remove excess buffer from sections.
5. Drop commercial HRP polymer (not provided with this product) that binds the primary antibody used before over the sections (enough to cover the tissue 50-100µl) and incubate in a humid chamber following the manufacturer's instructions.
6. Wash vigorously with PBS, agitate in PBS 1min.
7. Remove excess buffer from sections and apply commercial Diaminobenzidine (DAB) (not provided with this product) following the manufacturer's instructions.
8. Wash with water.
9. Counter stain the sections briefly (2-3secs) with Harris hematoxylin diluted 50% in distilled water.
10. Wash with distilled water, dehydrate and cover slip following normal laboratory protocols.

AENOR ER Gestión Calidad Productos Sanitarios UNE-EN ISO 13485 GS-0009/08
AENOR ER Empresa Registrada UNE-EN ISO 9001 ER-0051/08
AENOR Producto Certificado Proyecto I+D+I 068/0657/07

7°C
3°C

Emission date: 12/05/2014

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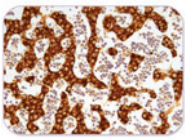
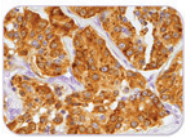


Alpha Fetal Protein (AFP)

Useful for the detection of primitive liver tumors and embryonic tumors of the gonads. RUO

Consists of a segment of single-stranded DNA complementary to expressed RNA with a length of 311 nucleotides.

5 Tests Item#: MDX2305

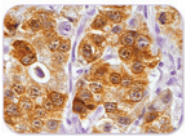


Calcitonin

Useful for the detection of Thyroid Medullary Carcinomas and C Cell Hyperplasia. RUO

Consists of two segments of single-stranded DNA complementary to expressed RNA, with lengths of 166 and 142 nucleotides.

5 Tests Item#: MDX1705

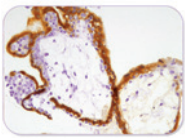


c-erbB2 (Her2/neu)

Useful for the detection of c-erbB2 (Her2/neu) RNA and its associated tumors. RUO

Consists of two segments of single-stranded DNA complementary to expressed RNA, with lengths of 672 and 1143 nucleotides.

5 Tests Item#: MDX2005

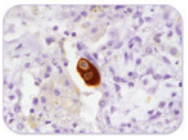


Chorionic Gonadotropin Subunit β (CGB)

Useful for the detection and localization of cells that produce comparable CGB hormone levels in choriocarcinomas as in germinal gonad and midline tumors. RUO

Consists of a segment of single-stranded DNA complementary to expressed RNA with a length of 278 nucleotides.

5 Tests Item#: MDX2505

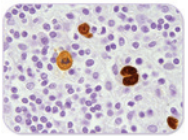


Cytomegalovirus (CMV)

Useful for the detection of cells infected by CMV in any location found, including: lymph node, central nervous system, retina, lung, and intestine. RUO

Consists of a single strand of DNA with a sequence length of 288 nucleotides. This sequence is complementary to CMV gene β2.7 mRNA.

5 Tests Item#: MDX1805

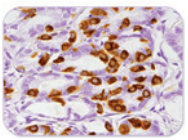


EBER

Useful for the detection of EBER 1+2 RNA in cells infected by the Epstein-Barr virus, both in reactive and tumoral cells. RUO

Consists of a fragment of single-stranded DNA of 526 nucleotides.

5 Tests Item#: MDX0105
20 Tests Item#: MDX0120



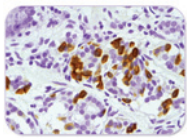
Gastrin

Useful for the detection of neuroendocrine tumors producing gastrin and validation of G cell hyperplasias in the stomach. RUO

Consists of a single-stranded DNA fragment with a length of 343 nucleotides targeted against gastrin mRNA.

5 Tests Item#: MDX1405

RUO = For Research Use Only. ***Not for use in diagnostic procedures.***

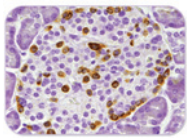


GHRL (Ghrelin/Obestatin)

Useful for the detection of cells producing the GHRL hormone and their tumors. RUO

Consists of a segment of single-stranded DNA with a length of 241 nucleotides, complementary to expressed RNA.

5 Tests Item#: MDX2105

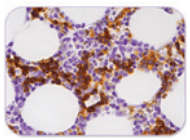


Glucagon

Useful for the detection of neuroendocrine tumors of the pancreas and digestive apparatus and their metastasis even in an absence of a clinically evident Endocrine Syndrome. RUO

Consists of a fragment of single-stranded DNA with a length of 413 nucleotides, complementary to expressed RNA.

5 Tests Item#: MDX1205

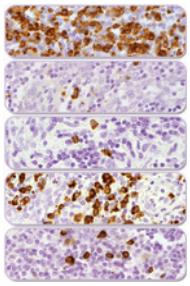


Hemoglobin Alpha Chain

Useful for the detection of erythroid cells in any tissue, as well as when its morphology is altered by some of the aforementioned pathological processes.

Consists of a fragment of single-stranded DNA with a length of 157 nucleotides, complementary to expressed RNA.

5 Tests Item#: MDX0205

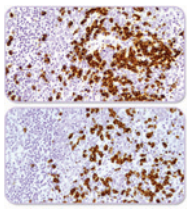


Heavy Chains (Alpha, Delta, Mu, Epsilon, Gamma)

Useful for the study of monoclonality in lymphoid tumors, lymphoproliferative syndromes, myelomas and for the study of immunodeficiency. RUO

Consists of a fragment of single-stranded DNA with a length of between 190 and 250 nucleotides, complementary to expressed RNA.

Type:	Item#:	Type:	Item#:
Alpha	MDX0605	Epsilon	MDX0805
Delta	MDX0705	Gamma	MDX0905
Mu	MDX1005	5 Tests	

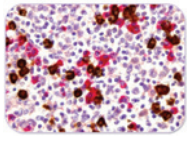


Light Chains (Kappa, Lambda)

Useful for the study of monoclonality in lymphoid tumors, immunodeficiency associated or idiopathic lymphoproliferative syndromes, and myelomas. RUO

Consist of a fragment of single-stranded DNA with a length of between 153 and 182 nucleotides, complementary to expressed RNA.

Type:	Item#:	Type:	Item#:
Kappa 5 Tests	MDX0405	Lambda 5 Tests	MDX0505
Kappa 20 Tests	MDX0420	Lambda 20 Tests	MDX0520

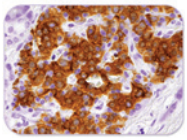


Light Chains, Dual Kappa-Lambda

Useful for the study of monoclonality in lymphoid tumors, immunodeficiency associated or idiopathic lymphoproliferative syndromes, and myelomas. RUO

Histosonda® Dual Kappa-Lambda consists of two fragments of single-stranded DNA with lengths of 153 and 182 nucleotides that are complementary to expressed RNA.

5 Tests Item#: MDX2705

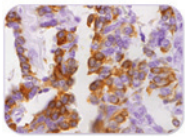


Insulin

Useful for the study and classification of neuroendocrine tumors of the pancreas and digestive apparatus and their metastasis even in an absence of a clinically evident Endocrine Syndrome. RUO

Consists of a fragment of single-stranded DNA with a length of 442 nucleotides, complementary to expressed RNA.

5 Tests Item#: MDX1105

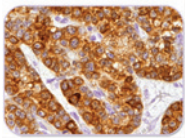


Pancreatic Peptide (PP)

Useful for the detection of pancreatic and extrapancreatic neuroendocrine tumors and their metastases. RUO

Consists of a single-stranded DNA fragment with a length of 238 nucleotides.

5 Tests Item#: MDX1905

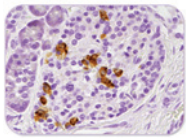


Serum Albumin

Useful for the detection of hepatocarcinomas in difficult situations (fine needle hepatic biopsies), and to distinguish them from metastatic tumors from other origins, as well as for diagnosing combined hepatocellularcholangicarcinoma (CHC). RUO

Consists of three segments of single-stranded DNA complementary to expressed RNA, with lengths of 305, 370 and 377 nucleotides.

5 Tests Item#: MDX0305

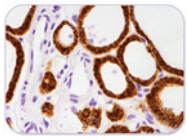


Somatostatin

Useful for the detection and classification of neuroendocrine tumors of the pancreas and digestive apparatus and their metastasis even in an absence of a clinically evident Endocrine Syndrome. RUO

Consists of a fragment of single-stranded DNA with a length of 302 nucleotides, complementary to expressed RNA.

5 Tests Item#: MDX1305

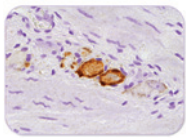


Thyroglobulin

Useful for the detection of thyroid tumors and their metastases. RUO

Consists of a segment of single-stranded DNA complementary to expressed RNA, with a length of 552 nucleotides.

5 Tests Item#: MDX1605



Vasoactive Intestinal Peptide (VIP)

Useful for the detection and classification of neuroendocrine tumors of the pancreas and digestive apparatus and their metastases even in an absence of a clinically evident Endocrine Syndrome. RUO

Consists of a single-stranded DNA fragment with a length of 533 nucleotides.

5 Tests Item#: MDX1505

RUO = For Research Use Only. ***Not for use in diagnostic procedures.***

XISH® Reagents

Proteinase K

Used for the deproteinization of formalin-fixed, paraffin-embedded tissue sections. RUO

5 Tests (1 Vial) Item#: MDA0105
20 Tests (4 Vials) Item#: MDA0120

Anti-Digoxin

Anti-Digoxin is used for revealing the probes. RUO

5 Tests (1 Vial) Item#: MDA0205
20 Tests (4 Vials) Item#: MDA0220

PBS 7.4

Liter Item#: BUP0350 / Gallon Item#: BUP0357

DAB Liquid Substrate System

Includes 70ml of buffer and 4ml of chromogen.

Item#: IMI04924E

Polymer HRP (Anti-Mouse)

Each bottle contains 17 ml and is sufficient for 340 test.

Item#: MDA0315

30% Hydrogen Peroxide

100ml Item#: SPH0426 / 500ml Item#: SPH0443

Steam Distilled Water

Liter Item#: AHW00192E / Gallon Item#: AHW00142E

Harris Hematoxylin

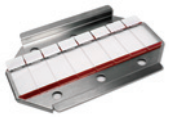
Pint Item#: HXHHEPT / Liter Item#: HXHHELT

XISH® Equipment

MyBath 4L - Digital Waterbath & Incubator

This compact 4 liter water bath features easy to use controls that allow the user to digitally select and monitor temperature. Its hinged lid provides a secure, covered environment and can be flipped open (to the rear) or completely and instantly removed at the user's option. Temperature Range: Ambient +5 to 100°C .

MyBath 4L Item#: EQW0101



XISH® Rail

The XISH© Rail slide rack is designed to convert the MyBath Digital Waterbath into a humidity chamber for probe hybridization in molecular diagnostics. The rack holds up to 8 slides at a time and sits just above the waterline when inserted into the waterbath. This allows precise slide incubation in a controlled humid environment.

XISH® Rail Item#: EQW0103

StainTray™ with Black Lid

StainTray™ is suitable not only for routine staining requiring a humid chamber, but is ideal for hematology, cytology and microbiology laboratories.

StainTray™ 10 Slide Tray Item#: LWS10BK
StainTray™ 20 Slide Tray Item#: LWS11BK

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