

# EXPRESS ISH<sup>®</sup>

Express In-Situ Hybridization

**The Best Way to Bring  
Molecular Diagnostics  
into your lab!**



American MasterTech  
scientific laboratory supplies

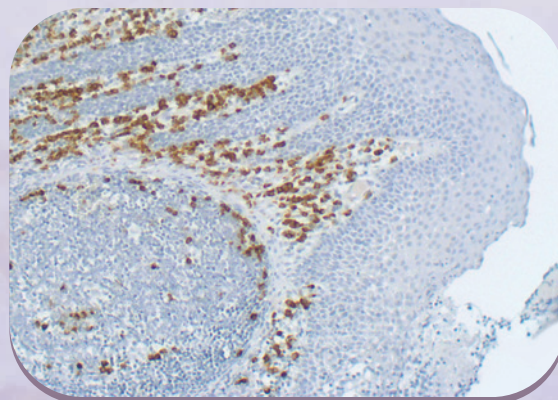
**With any laboratory oven and these items shown below,**



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

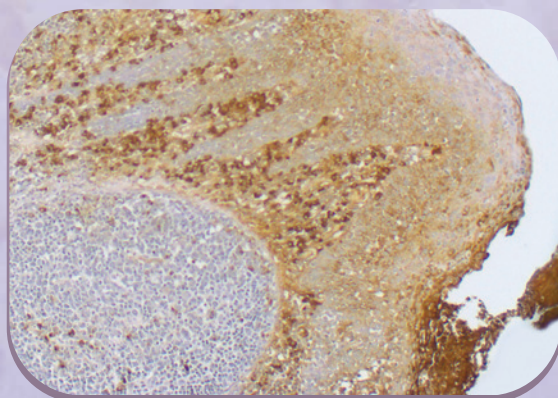
**No expensive equipment,  
and no toxic chemicals  
like formamide are required!**

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



Kappa-Light Chain XISH<sup>®</sup> Probe

***instead of this....***



Kappa-Light Chain IHC Antibody

# 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.



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## HISTOSONDA CHORIONIC GONADOTROPIN SUBUNIT B (CGB)

Assay with one vial for 5 individual reactions. 65µl for reaction.

Product classification:  
For Research Use only (RUO)

### INTRODUCTION

Human chorionic gonadotropin is a glycoprotein produced by the trophoblastic cells of the placenta generally 10 to 12 days after conception, its principle function is to stimulate the corpus luteum to continuously produce progesterone which stimulates the secretory differentiation of the endometrium.

Chorionic Gonadotropin belongs to a family of glycoproteins that includes luteinizing hormone, follicle stimulating hormone and the pituitary thyrostimulating hormone. Each one of these hormones consists of two chains denominated  $\alpha$  and  $\beta$ . The  $\alpha$  chain is the same in the 4 hormones but each one differs in its  $\beta$  chain.

Histosonda Chorionic Gonadotropin  $\beta$  Subunit consists of a fragment of single stranded DNA of 278 nucleotides that is targeted against the  $\beta$  chain of human chorionic gonadotropin RNA.

There are antibodies against the  $\beta$  subunit of chorionic gonadotropin but they generally produce a strong tissular background that makes the histological interpretation difficult.

The germinal tumors of the gonads and the midline can demonstrate distinct differentiation levels that are difficult to interpret with conventional histological techniques.

Histosonda Chorionic Gonadotropin  $\beta$  Subunit permits the detection and localization of cells that produce this hormone as much in choriocarcinomas as in germinal gonad and midline tumors.

### INTENDED USE

For Research Use only.

Not for diagnostic procedures.

Histosonda CGB is useful for the detection and localization of cells that produce this hormone as much in choriocarcinomas as in germinal gonad and midline tumors.

### WARNINGS AND PRECAUTIONS

Histosonda CGB has been designed for professional research use 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 ( $\text{NaN}_3$ ). 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

1 tube with the necessary amount of Histosonda CGB for developing 5 single reactions.  
Histosonda CGB consists of a segment of single-stranded DNA complementary to expressed RNA with a length of 279 nucleotides. This probe has been labeled with Digoxigenin.

### STORAGE CONDITIONS

Supplied reagents are stored at 3-7°C 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 Chorionic Gonadotropin Subunit  $\beta$  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.

### ASSAY LIMITATIONS

Histosonda CGB has been optimized to detect Chorionic Gonadotropin Subunit  $\beta$  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.

### 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

Before starting pre-heat the humid chamber to 62°C.

#### 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%  $\text{H}_2\text{O}_2$  or 3%  $\text{H}_2\text{O}_2$  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 55°C 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 com-

pletely covered and avoiding air bubbles.

3. Place the slides in a horizontal position in a humid chamber and close well.
4. Incubate at 62°C for 1hr.

#### 4. Washing the probe

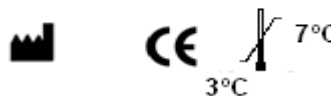
**! The probe solution is very viscous. It is very important to wash the slides well to ensure the probe solution is completely removed.!**

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 distilled 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.



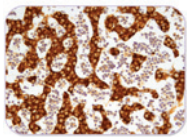
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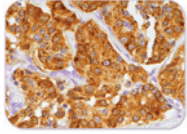
### Alpha Fetal Protein (AFP)

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



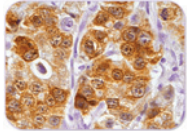
### Calcitonin

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



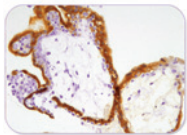
### c-erbB2 (Her2/neu)

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



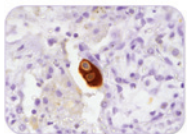
### Chorionic Gonadotropin Subunit $\beta$ (CGB)

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



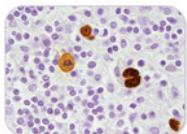
### Cytomegalovirus (CMV)

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  $\beta$ 2.7 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

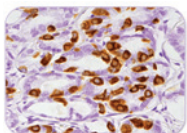
Consists of a fragment of single-stranded DNA of 526 nucleotides.

5 Tests

Item#: MDX0105

20 Tests

Item#: MDX0120



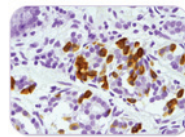
### Gastrin

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



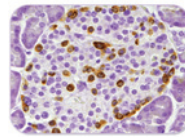
### GHRL (Ghrelin/Obestatin)

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



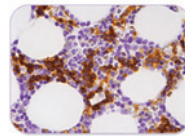
### Glucagon

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



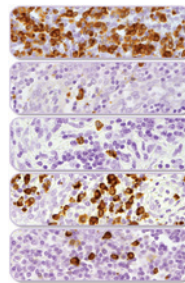
### Hemoglobin Alpha Chain

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

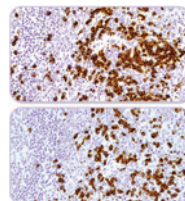


### Heavy Chains (Alpha, Delta, Mu, Epsilon, Gamma)

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	

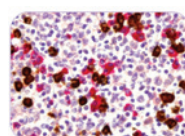


### Light Chains (Kappa, Lambda)

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



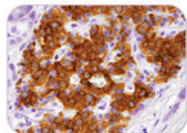
### Light Chains, Dual Kappa-Lambda

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

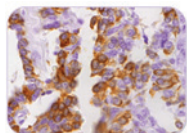


#### Insulin

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

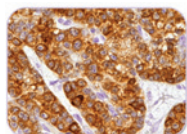


#### Pancreatic Peptide (PP)

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

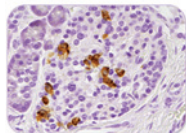


#### Serum Albumin

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 hepatocellular-cholangiocarcinoma (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

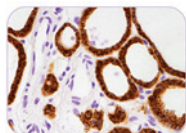


#### Somatostatin

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

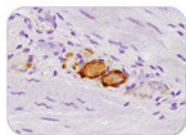


#### Thyroglobulin

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

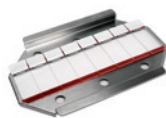
## XISH® Equipment



#### 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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