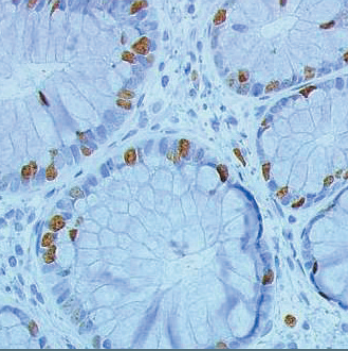
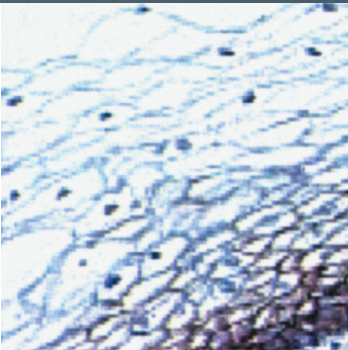
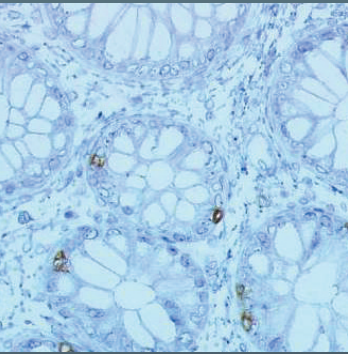


A reliable, time tested solution to unmask antigen on formalin-fixed sections

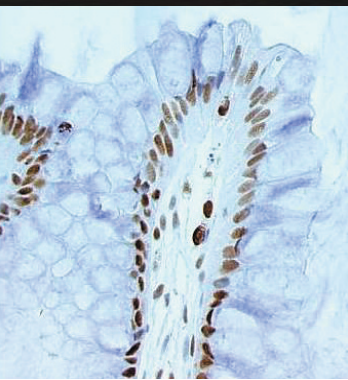


Retriever 2100

For Antigen
Unmasking



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Retriever 2100

For Antigen Unmasking

A reliable, time tested solution to unmask antigen on formalin-fixed sections

Overview

Our Unique Retriever solves the problem of staining formalin fixed tissues. This is an affordable solution to all known major problems with immuno-histochemistry on paraffin sections. Ease of use combined with high reproducibility of the results will give you the best quality immunostaining.

The Retriever is a bench-top model for thermally processing slides of formalin-fixed, paraffin embedded tissues prior to immunostaining. The model has been designed to ensure identical processing of all the samples during a processing cycle, as well as the identical processing of the samples in individual sessions. The retriever preserves processed tissues.

Now you can:

- Run antigen unmasking in 6 various buffers at once.
- Perform gentle antigen retrieval that does not damage the tissue morphology.
- Get identical results every time.
- Compare series of slides treated in independent sessions.

How does the Retriever work?

Basically it is a pressure cooker. However, a pressure cooker specially designed to unmask the antigen on tissue sections. The core principle is heating of the chambers with the slides at high temperature ($>120^{\circ}\text{C}$) and high pressure. Sensors control the heating profile for the temperature and pressure to be reached at certain pace and over certain time. We did a lot of tests to find the optimal settings. When the required temperature is reached, it will be kept for several minutes. After that the slides will be cooling over 2 hours. Specially designed thermal walls of the unit control the speed of cooling of the inner chamber and slides

Who would benefit from using our Retriever?

- Investigative Pathology, where the high quality of staining (a picture may be published!) is required.
- Any labs that is short on technical personnel: any student or post-doc can process slides for the staining without using much time
- Small routine pathology labs, where a limited number of slides should be processed daily
- Anyone who uses highly valuable samples, such as tissue arrays or unique tissue samples: Simplicity and reliability of the unit ensures the safety of your sample, and a high quality antigen unmasking

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How to use:

Step 1.

Deparaffinized sections on slides are placed into the Processing (Tissue Slide) Chambers. Retriever can accommodate simultaneously from 1 to 6 Chambers, which allows you to process a series of slides in up to 6 different antigen unmasking buffers within the same session. Fill the chambers with a buffer of your choice.

Step 2.

Place the Chambers into the Rack.

Fill the Retriever with 750 ml of deionized/distilled water. Place the Rack into Retriever.

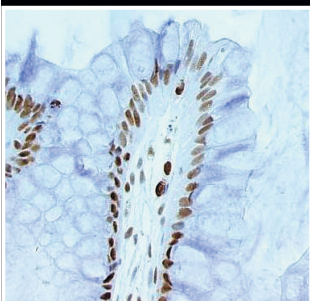
Close the lid by a simple twist.

Step 3.

Push the Start button. The tissues will be processed automatically. In about two hours (depends on the load) you can open the lid and proceed immunostaining.

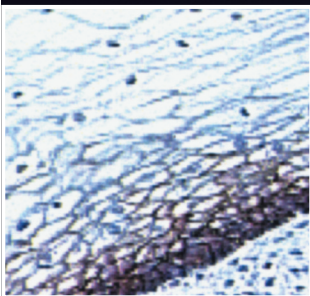


Examples of Staining



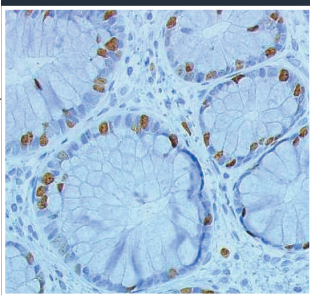
PCNA (Nuclear antigen)

Sections of formalin-fixed, paraffin embedded tissue of human duodenum were deparaffinized and processed in Retriever to unmask the antigen. We used one cycle in R-Buffer C (pH 6.0) with cooling of the slides overnight. Antibody PC-10 against proliferation marker PCNA (nuclear) was used together with R-Detect HRP detection system for immunostaining of sections.



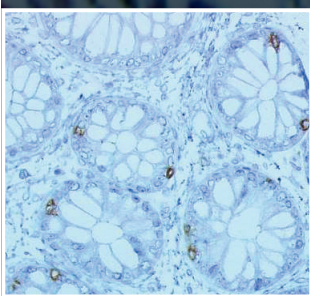
E-cadherin (Membranous, extracellular domain)

Sections of formalin-fixed, paraffin embedded tissue of human cervix were deparaffinized and processed in Retriever to unmask the antigen. We used one cycle in R-Buffer C (pH 6.0) with cooling of the slides overnight. Antibody HECD-1 against cell adhesion molecule E-cadherin was used together with R-Detect HRP detection system for immunostaining of sections.



Ki-67 (Nuclear antigen)

Sections of formalin-fixed, paraffin embedded tissue of human sigmoid were deparaffinized and processed in Retriever to unmask the antigen. We used one cycle in R-Buffer C (pH 6.0) with cooling of the slides overnight. Antibody MIB-1 against proliferation marker Ki-67 (nuclear) was used together with R-Detect HRP detection system for immunostaining of sections.



CD8 (Membrane)

Sections of formalin-fixed, paraffin embedded tissue of human sigmoid were deparaffinized and processed in Retriever to unmask the antigen. We used one cycle in R-Buffer A (pH 8.1) with cooling of the slides overnight. Antibody #4B11 against T-cell marker CD8 (cell membrane) was used together with R-Detect HRP detection system for immunostaining of sections.

SPECIFICATIONS

Height	335 mm
Capacity	9 liters
Max. Instrument length	228 mm
Width	340 mm
Net Weight	4.5 kilos
Internal Chamber Dimensions (d/h)	210/230 mm
Max. Load Weight	3.0kg

Other Specifications:

Fuses — Located under the control module, fuses F1 0A, 32 x 6.3mm, ceramic sand filled, Mains plug top fuse (User replaceable), F1 3A to BS1362 UK ONLY.

Rating — Models are rated continuously for intermittent use.

Body — Deep drawn aluminum. Lid - Aluminum.

Heater — Externally surface mounted mechanically fixed electric element.

Temperature Cut Out — Thermal fuse.

Pressure — Calibrated pressure release valve.

Max. Single Fault Temperature — 133.3°C

Environment Conditions — indoor use - temperature 5°C to 40°C - altitude up to 2000m - maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C. - mains supply voltage fluctuations not to exceed +10% of the nominal voltage

Input Connections — Mains inlet socket 'hot' format conforming to IEC 302.

Safety Shut Down — See "Temperature Cut Out".

Choosing A Buffer:

If you already know the buffer that can be used for Microwave treatments of sections in order to unmask you antigen of interest, the high chances are that the same buffer may be used in Retriever.

To improve the morphology of tissue of the processed sections, use one of our supplied ready-to-use buffers by choosing them according to pH.

We have commercially available the following buffers:

- R-Buffer A pH 6.0
- R-Buffer B pH 8.0
- R-Buffer C pH 4.5
- R-Buffer U pH 6.0
- G variants of the same buffers for a more gentle processing of the tissues.

To successfully retrieve the antigen of interest on fixed sections, please remember that two factors define the choice of buffer:

- The nature of the antigen/epitope and of the antibody used for its detection
- The fixative used and the degree of fixation.

Correction for Fixative & Degree of Fixation

The suggested protocol for processing tissues is optimized for routinely formalin-fixed and paraffin embedded material. If the tissue used for sections was insufficiently fixed, overfixed (was left in formalin for too long), or other fixative was used, the protocol may require some modifications.

The easiest correction is to use EMS's own specially formulated buffers for a gentle (G) tissue processing. Use them instead of the basic buffers. For overfixed material try using U buffer or run the additional cycle in the same buffer.

Fixative Used on Tissues	Buffer
Formalin, buffered	R-Buffer A, B, C or U
Formalin-Zn	R-Buffer A, B, C or U
Mirsky fixative	Under testing
Boonfix I	Under testing
Boonfix II	Under testing
PLP	R-buffer AG, BG, or CG

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Ordering Information

Choosing Buffer: Correction for the Nature of the Antigen and Epitope.

The choice of the buffer depends on the nature of the antigen and the location of the epitope of interest. We advise you to first run the test for the most appropriate buffer using the tissues where the expression of the antigen does occur. General guidelines for choosing the buffer:

Most of the nuclear antigens (apoptosis-related, survival-related, proliferation-related) R: Buffer A (or AG).

Cell adhesion molecules, cell membrane antigens (extracellular domain) R: Buffer A (or AG)

Cytoskeleton and cytoskeleton-associated molecules – R: Buffer A (or AG)

Intracellular domain of some adhesion molecules and surface receptors – R: Buffer B (or BG)

Intracellular domain of some adhesion molecules and surface receptors – R: Buffer C (or CG)

Most of antibodies raised against a linear peptide – R: Buffer U (or UG)

Cat. No.	Description	Qty.
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Retriever 2100

62700-20	Retriever 220 volt	each
62700-10	Retriever 115 volt	each

Buffers and Accessories

62706-10	R-Buffer A (10x)	250 ml
62706-11	R-Buffer B (10x)	250 ml
62706-12	R-Buffer C (10x)	250 ml
62706-13	R-Buffer U (10x)	250 ml
62707-10	R-Buffer AG (2x)	250 ml
62707-11	R-Buffer BG (2x)	250 ml
62707-12	R-Buffer CG (2x)	250 ml
62707-13	R-Buffer UG (2x)	250 ml
62705-01	Slide Chamber	3/pk
62705-02	Chamber Rack	each
62705-03	Lifting Device	each
62705-04	Cord Set (UK)	each
62705-05	Cord Set (Europe)	each
62705-06	Cord Set (US)	each
62705-07	Green Silicone Sealing Gasket	each

Retriever Tissue Slides

Retriever Tissue Slides are high adhesive glass microscope slides for paraffin-embedded tissue section, including tissue arrays. The slides were designed and optimized for the EMS Antigen Retriever (62700 series) to preserve the section attachment and tissue morphology during heat-induced epitope recovery in Retriever in pH6 (Low), pH9 (High), or Universal Retriever buffer.

Slides can also be successfully used in any other epitope-recovery technology (autoclave, microwave, proteolytic) and show superior performance over positively charged or polylysine-coated slides, especially in high pH buffers and recovery buffers containing EDTA.

General Properties

- Retriever Tissue Slides are made from soda lime glass
- Dimensions approximately 76 x 26mm, Thickness 1.0mm.
- 90° ground edges
- Colored or white marking area 20mm, at one end, on one side
- Pre-cleaned, ready for use
- Autoclavable
- Supplied in plastic boxes of 72 pieces (call for carton price, 20 boxes in a carton)
- Carry recommended best before date and batch number for comprehensive information and traceability

Cat. No.	Description	Qty.
71880-50	Retriever Tissue Slides	72/pk
71880-50-CS	Retriever Tissue Slides	20/pk case

R-UNIVERSAL Epitope Recovery Buffer (10x stock)

Why the Universal Buffer?

Epitope recovery on formalin-fixed, paraffin embedded tissue sections, requires heat-induced treatment in buffer or, sometimes, proteolytic treatment of the deparaffinized tissue section. Which buffer to use, greatly depends on the exact antibody and the properties of the recognized epitope, therefore, one can find in literature and practice use of many buffers, including Citrate pH 3.4, Citrate 6.0, EDTA 8.0, Tris 9.0- 10.0, Tris-EDTA, etc.



Moreover, for individual antigens also the time of recovery in the individual buffer should be defined, as this may be different, and the treatment often destroys the epitope.

EMS offers a novel (patent-pending) technology for epitope recovery, primarily based on reversing the fixation effects of formaldehyde, which created links primarily between μ -amino groups of lysine and other amino-groups.

The buffer was extensively tested in pathology Departments in United Kingdom, and has shown excellent results when used with different antibodies, including those that normally require for successful staining treatment only in Low, or only High pH buffers, or require proteolytic treatment.

The EMS Universal Buffer may be used in any heat-induced epitope recovery system (the time and temperature of treatment should be tested), but was specifically adjusted for tissue sections processing in our Antigen Retriever (62700 Series).

Using our Universal Buffer in our Retriever guarantees the highest rate of success in recovering epitope for any antibody, especially one that was never previously used on formalin-fixed sections.

Properties

Clear, non-toxic solution.

Presentation

R-Universal Buffer is supplied as 10x concentrate. For epitope recovery dilute 1 part of stock with 9 parts of deionized water.

Application

For epitope recovery dilute 1 part of stock with 9 parts of deionized water.

Stability and Storage

The preparation is stable for 1 year when stored unopened at +4°C. Every lot is issued with a certificate indicating the expiry date.

After opening, store at +4°C in the refrigerator and use within 6 months.

Certification

Each lot is certified for compliance to specifications. The product is produced under DIN EN ISO 9001 :2008 Quality Management system for the products in Immunoassay Development and Measurement, Products for Bioanalytics and Immunoassays.

	Cat. No.	Description	Qty.
0-4°C	62719-10	R-UNIVERSAL Epitope Recovery Buffer (10x stock)	125 ml
0-4°C	62719-20	R-UNIVERSAL Epitope Recovery Buffer (10x stock)	500 ml

ImmunoSaver Antigen Retriever

ImmunoSaver allows for Immunostaining with quick and easy activation of cell membranes and the nucleus. ImmunoSaver provides efficient antigen retrieval for successful immunostaining of a wide variety of antigens under optimized conditions.

Protocols for both Light and Electron Microscopy may be found with this reagent.



Cat. No.	Description	Qty.
64142	ImmunoSaver	100 ml

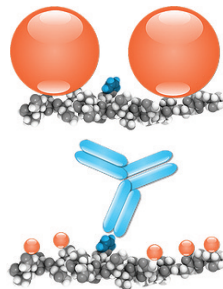
Reduce the Background, Not the Specific Staining

Reliable time tested solution to unmask antigen on formalin-fixed sections

Any antibody preparation has some potential to produce non-specific reaction in the assay. This originates from:

- non-specific antibodies that are present in some proportion in any polyclonal antibody preparation, including affinity purified ones (often "affinity purified" means only isolation of IgG fraction on Prot A/G column, not the purification on the antigen column under very stringent conditions)
- low specificity antibodies among specific ones in polyclonal
- fragments of fallen apart IgGs in stored preparations, including monoclonal
- separate heavy and light chains of specific antibodies, produced by most hybridomas

All these are capable of binding non-specifically to molecules on tissue sections, blots, fixed cells and other objects for immune detection. In case of retrieved formalin sections the risk of non-specific reaction is even increased, since to activate the epitope recovery the proteins comprising the tissue sections are denatured during HIER, thus making accessible many domains that are charged and are capable of binding the test immunoglobulins on non-specific manner.



The standard means to block non-specific binding of specific antibody preparation is to add some irrelevant protein, such as BSA, other serum, casein, etc. However, everyone who tried to do this knows that increasing (for effective blocking) concentration of such blocking agent leads to a great reduction of specific reaction as well. This is due to large blocking molecules binding to accessible sites on section and thus sterically blocking access of specific antibodies to epitopes of interest (schematically represented in the figure on the left, top). All our buffers developed for immune assays contain instead short (0.6-2 kD) peptides that are capable of block effectively non-specific reaction while not affecting the specific binding of antibody.

The presented collection of Immunohistology buffers has also some other benefits (*see back page*) and allows you to achieve the best quality IHC result without compromising the antigen detection. The buffers can also be used in other immune assays, such as immunofluorescence on sections, flow cytometry on fixed cells, western blot, hybridization of sections with antibody detection.

The Retriever IHC Buffers empower you to control non-specific staining on every step of immunohistochemistry. They are especially highly recommended for research pathology where, in contrast to diagnostics, many polyclonal and/or low-affinity antibodies are used.

All buffers available in 50 ml, 125 ml and 500 ml package. Ready to use.

Section Block

A new class of blocking solutions based on chemically modified and fragmented ultra-pure casein. Effectively reduces unwanted binding of primary antibody and conjugates you use to charged surface of the slide and tissue section. Greatly reduces non-specific binding while preserving the specific reaction, by saturating potential non-specific protein-protein interactions. Moreover, in contrast to BSA-based, IgGm casein or serum-based blocking solutions, there is no interaction of specific antibody and blocking protein itself. Therefore, it is not comparable to other commercially available or home-made blocking solutions. Recommended for research and diagnostic pathology, especially for retrieved sections and polyclonal antibodies.



Cat. No.	Description	Qty.
62710	Section Block	50 ml
62711	Section Block	125 ml
62712	Section Block	500 ml

Antibody Diluent

Buffer for diluting your primary and secondary antibodies, especially if they were stored for a while, even at -20 in glycerol, or in refrigerator. Nonspecific binding of the antibodies, negative effects of disturbing substances and low or medium affinity cross-reactivities of the antibodies will be minimized, making your result more reliable. Excellent for IHC (frozen and formalin sections), flow cytometry on fixed cells, Western Blot and other immune assays.



When used in pathology, it also greatly reduces non-specific reactivity of human serum components and immunoglobulins in tissue, vessels and cells with mouse antibodies used on section.

For especially "trouble"-giving antibodies, as well as for in situ PCR applications, this diluent may also be used as a washing buffer, preventing secondary binding of your analytes during washing.

Cat. No.	Description	Qty.
62713	Antibody Diluent	50ml
62714	Antibody Diluent	125ml
62715	Antibody Diluent	500ml
62713-01	Antibody Diluent for Frozen Sections	50ml
62714-01	Antibody Diluent for Frozen Sections	125ml
62715-01	Antibody Diluent for Frozen Sections	500ml

HRP-Conjugate Diluent

Specifically designed for preparing solution of your HRP-conjugate used as the detection reagent. It is the Antibody-diluent buffer with additional component for stabilizing your HRP-conjugate. Allows you to further standardize the assay preparing ready-to-use conjugate solutions in advance and store them in refrigerator without loss of activity.



Cat. No.	Description	Qty.
62716	HRP Conjugate Diluent	50 ml
62717	HRP Conjugate Diluent	125ml
62718	HRP Conjugate Diluent	500 ml

Slide Washing Buffer 10 X

Cat. No.	Description	Qty.
62718-15	Slide Washing Buffer 10 X	500 ml

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IHC Antigen Retrievers

I. Trypsin Reagent

Description: Trypsin is used for proteolytic digestion of formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies. In IHC most commonly used fixative like formalin; mask tissue antigens (cellular, membrane and nuclear) by cross-linking process, this results in poor or no staining in IHC. Trypsin digestion improves immunoreactivity of some antigens in FFPE tissue sections. For cytokeratin clone AE3 and AE1/AE3, this enzyme works much better than boiling the tissue with citrate buffer.

Storage: 2–8°C. **DO NOT FREEZE**

Intended Use: Use as antigen retriever for some antibodies in IHC. Please refer to primary antibody protocol for IHC.

Reagent: This enzyme is supplied as concentrated form along with buffer. The kit comes in 2 sizes. Small: Reagent B (Buffer) is 15 ml and the Reagent E (Enzyme) is 2 ml

Large: Reagent B (Buffer) is 100 ml and the Reagent E (Enzyme) is 10 ml

Cat. No.	Description	Qty.
64142-01	Trypsin Kit Small	Kit
62142-02	Trypsin Kit Large	Kit

II. Pronase Reagent, Ready To Use

Description: Pronase is used for proteolytic digestion of formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies. In IHC most commonly used fixative like formalin mask tissue antigens (cellular, membrane and nuclear) by cross-linking process, this results in poor or no staining in IHC. Pronase digestion of FFPE tissue section improves accessibility of antibodies to tissue antigens.

Storage: 2–8°C. **DO NOT FREEZE**

Intended Use: Use as antigen retriever for some antibodies in IHC. Please refer to primary antibody protocol for IHC.

Reagent: Ready-to-use Pronase reagent available in 2 sizes: 15 and 100 ml.

Cat. No.	Description	Qty.
64142-03	Pronase Reagent	15 ml
62142-04	Pronase Reagent	100 ml

III. Pepsin Reagent, Ready To Use

Description: Pepsin is used for proteolytic digestion of formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies. In IHC most commonly used fixative like formalin mask tissue antigens (cellular, membrane and nuclear) by cross-linking process, this results in poor or no staining in IHC. Pepsin digestion of FFPE tissue section improves accessibility of antibodies to tissue antigens.

Storage: 2–8°C. **DO NOT FREEZE**

Intended Use: Use as antigen retriever for some antibodies in IHC. Please refer to primary antibody protocol for IHC.

Reagent: Ready-to-use Pepsin reagent available in 2 sizes: 15 and 100 ml

Cat. No.	Description	Qty.
64142-05	Pepsin Reagent	15 ml
62142-06	Pepsin Reagent	100 ml

IV. Citrate Buffer pH 6.0 (10x)

Description: This buffer is intended for heat-induced antigen retriever on formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies. In IHC most commonly used fixative like formalin mask tissue antigens (cellular, membrane and nuclear) by cross-linking process, this results in poor or no staining in IHC. The use of this buffer on FFPE tissue section improves accessibility of antibodies to tissue antigens.

Storage: 2–8°C. **DO NOT FREEZE**

Intended Use: 1X buffer solution is intended for heat-induced antigen retriever in IHC. Please refer to primary antibody protocol.

Reagent: 10 X, pH 6 antigen retriever solution.

Preparation of Working Solution: Dilute this 10X buffer as needed (e.g. 90 ml of deionized or distilled water + 10 ml of this buffer), mix well, 1X buffer can be stored at 2–8°C

Cat. No.	Description	Qty.
64142-07	Citrate Buffer pH 6.0	100 ml
62142-08	Citrate Buffer pH 6.0	1000 ml

V. EDTA Buffer pH 8.5 (10x)

Description: This buffer is intended for heat-induced antigen retriever on formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies. In IHC most commonly used fixative like formalin mask tissue antigens (cellular, membrane and nuclear) by cross-linking process, this results in poor or no staining in IHC. The use of this buffer on FFPE tissue section improves accessibility of antibodies to tissue antigens.

Storage: 2–8°C. **DO NOT FREEZE**

Intended Use: 1X buffer solution is intended for heat-induced antigen retriever in IHC. Please refer to primary antibody protocol.

Reagent: 10 X, pH 8.5 antigen retriever solution.

Preparation of Working Solution: Dilute this 10X buffer as needed (e.g. 90 ml of deionized or distilled water + 10 ml of this buffer), mix well, 1X buffer can be stored at 2–8°C

Cat. No.	Description	Qty.
64142-09	EDTA Buffer pH 8.5	100 ml
62142-10	EDTA Buffer pH 8.50	1000 ml

VI. Tris Buffer pH 10.0 (10x)

Description: This buffer is intended for heat-induced antigen retriever on formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies. In IHC most commonly used fixative like formalin mask tissue antigens (cellular, membrane and nuclear) by cross-linking process, this results in poor or no staining in IHC. The use of this buffer on FFPE tissue section improves accessibility of antibodies to tissue antigens.

Storage: 2–8°C. **DO NOT FREEZE**

Intended Use: 1X buffer solution is intended for heat-induced antigen retriever in IHC. Please refer to primary antibody protocol.

Reagent: 10 X, pH 10 antigen retriever solution.

Preparation of Working Solution: Dilute this 10X buffer as needed (e.g. 90 ml of deionized or distilled water + 10 ml of this buffer), mix well, 1X buffer can be stored at 2–8°C

Cat. No.	Description	Qty.
64142-11	Tris Buffer pH 10.0	100 ml
62142-12	Tris Buffer pH 10.0	1000 ml

VII. ImmunoHistoZyme™, Ready-to-Use

Description: ImmunoHistoZyme is used for proteolytic digestion of formalin-fixed paraffin-embedded (FFPE) tissue sections prior to application of antibodies. In IHC most commonly used fixative like formalin mask tissue antigens (cellular, membrane and nuclear) by cross-linking process, this results in poor or no staining in IHC. ImmunoHistoZyme digestion of FFPE tissue section improves accessibility of antibodies to tissue antigens.

Storage: 2–8°C. **DO NOT FREEZE**

Intended Use: Use as antigen retriever for some antibodies in IHC. Please refer to primary antibody protocol for IHC.

Reagent: Ready-to-use ImmunoHistoZyme Reagent.

Cat. No.	Description	Qty.
64142-13	ImmunoHistoZyme	15 ml
62142-14	ImmunoHistoZyme	100 ml

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