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# **IHC Enzyme Antigen Retrieval Reagent**

Catalog number: AR0022

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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Catalog Number: AR0022, Storage: Store at 4°C. Avoid multiple freeze-thaw cycles.

#### **Overview**

Physical State	Slightly viscous liquid
Pack Size	50 mL (for 500-750 assays)
Form Supplied	Solution
Content	Multiple proteinases
Composition	Proteolytic Enzymes
Volume of Final Solution	50 mL
Enzymatic Activity	Protease
Storage	Upon receipt store at 4°C. It is stable for one year.
Equivalent	N/A
Туре	Buffers and Mixes
Research Area	Molecular Biology, Cell Biology, Histology
Applications	IHC
	PIER/EIER
	PEFF tissues
Cite This Product	IHC Enzyme Antigen Retrieval Reagent (Boster Biological Technology,
	Pleasanton CA, USA, Catalog # AR0022)
Precautions	FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC AND CLINICAL
	USE

## **Assay Principle**

IHC enzyme antigen retrieval reagent is a ready to use proteolytic enzyme solution intended to be used as a pretreatment reagent in IHC for enzymatic digestion of tissues employed in proteolytic induced epitope retrieval (PIER) techniques applied upon paraffin-embedded formalin-fixed (PEFF) tissue sections on slides following deparaffinization and prior to antibody staining.

## Background

The IHC Enzyme Antigen Retrieval Reagent contains multiple proteinases that catalyze proteolysis in tissue samples, breaking the proteins into smaller peptide fractions and amino acids. Enzymatic digestion is commonly used in IHC in the PIER method for antigen retrieval to overcome the loss of immunoreactivity in epitopes caused by the covalent cross-links (methylene bridges) that are formed in tissues during formalin fixation. Proteolysis serves to improve the accessibility of antibodies to target antigens by two main mechanisms: by increasing the penetration of immunoreagents into tissue structures as a result of both tissue digestion and relaxation of the protein structure rigidity, and by restoring the methylene-bridged epitopes. Since proteases exhibit different cleavage site specificity, the combination of multiple proteinases provides a broader spectrum of proteins digestion in a tissue sample. Thus various types of antigenic targets (e.g. protein, glycoprotein, or non-protein, inter- or intracellular antigens) can benefit from this cocktail of digestion enzymes.

## Usage and Handling

• The use of enzyme digestion method may destroy some epitopes and tissue morphology. In these cases HIER methods for antigen retrieval should be used.

• When using the enzymatic digestion reagents it is important to not over or under digest. Preliminary experiments should be conducted to



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determine the optimum incubation time, temperature, and enzyme concentration.

• In general, enzymatic methods are shown to improve IHC staining results less than HIER and are much less reproducible. Their use is primarily recommended for difficult-to-retrieve epitopes when HIER fails to produce satisfactory results.

• IHC Enzyme Antigen Retrieval Reagent is for scientific laboratory research purposes and is not for diagnostic, therapeutic, prophylactic or in vivo applications.

### **Assay Protocol**

1. Add IHC enzyme antigen retrieval reagent directly onto the tissue or cell, and cover the entire tissue or cell completely.

2. Incubate at 37°C for 5-10 minutes.

3. Wash the tissue or cell with PBS.

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