

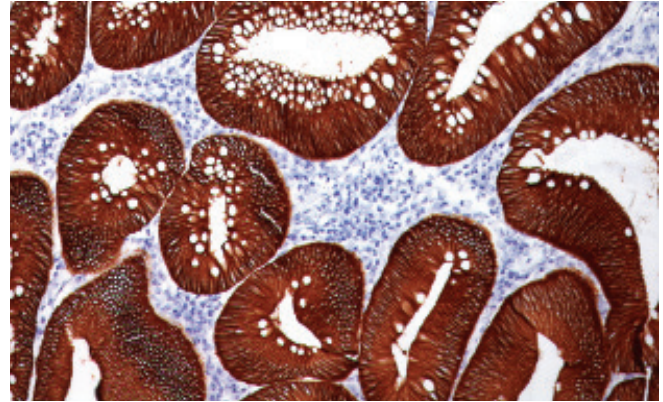
The Breakdown: Enzymes and Epitope Retrieval

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Tissue fixation can lead to protein cross-linking, which can block antigenic sites and limit antigen-antibody binding. To break these cross links and expose the antigen's epitope, antigen retrieval will be necessary. The two most common methods to recover masked epitopes are heat induced epitope retrieval (HIER) and proteolytic antigen retrieval (PIER).

PIER relies on enzymes, such as trypsin and pepsin, to catalyze proteolysis, breaking down proteins into smaller peptide fractions and amino acids, thus unmasking antigens and restoring antigenicity. Different enzymes cleave at different amino acids. Therefore, to identify the ideal retrieval method for a specific antigen, it is best practice to test one or two enzymes, along with HIER. Certain antigens, such as cytokeratins and immunoglobulins¹, may even benefit from a combination of enzyme and heat retrieval.

PIER is particularly useful for proteins that are difficult to retrieve or when HIER produces less than satisfactory results. Enzymes may be used at room temperature or 37°C and, while incubation time may vary, 10-15 minutes is typical. Excess retrieval should be avoided as it can destroy tissue morphology and even some epitopes. Since PIER tends to be less reproducible than HIER, optimization to determine the enzymes optimal concentration and incubation time and temperature is of utmost importance.



Pepsin digestion was utilized with MOC-31 antibody on colon cancer. Amyloid P, Cytokeratin 17, and Ber-EP4 are a few other of Biocare's antibodies that recommend pepsin digestion.

Products for Enzymatic Antigen Retrieval

Enzyme	Catalog Number	Description
Carezyme I: Trypsin Kit	TRP955	Two component, ready-to-use solution; can be used at different concentrations. The buffer incorporates a color-coded pH indicator that enables the end-user to visually inspect the solution and see that the mixture or buffer is at the proper pH. Stable for 5 working days at 2-8°C.
Carezyme II: Pepsin	PEP956	Single-component, ready-to-use solution Can be used manually or on an automated staining system
Carezyme III: Pronase Kit	PRT957	Two-component system and can be used at different concentrations. An aggressive enzyme which can be used at room temperature or at 37°C. Certain antibodies, such as EGFR, require this type of aggressive enzyme digestion. The buffer incorporates a color-coded pH indicator that enables the end-user to visually inspect the solution and see that the mixture or buffer is at the proper pH. Can be used manually or on an automated staining system. Stable for 7 days at 4°C.
Protease XXIV	PR960KG15	Lyophilized digestive enzyme for IHC pretreatment Stable for 5 working days at 2-8°C.

To learn more about Biocare Medical's enzymes and other antigen retrieval reagents and instrumentation, please call 800-799-9499 or visit the following website links: <https://biocare.net/products/ancillaries/enzymes/>; <https://biocare.net/products/ancillaries/antigen-retrieval-solutions/>; <https://biocare.net/products/instrumentation/decloaking-chamber-nxgen/>

1. Immunohistochemical Staining Methods, Sixth ed. Dako Denmark A/S, An Agilent Technologies Company, 2013.