WHITEPAPER

Antigen Retrieval Terms for IHC

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Antigen retrieval (AR) is a fundamental part of any immunohistochemistry (IHC) protocol. However, with so many different names for this one process, it can be difficult to recognize what an individual protocol or paper is referring to. Why is it sometimes called "retrieval" and other times simply "pretreatment?" Is ER the same as AR? What about HIER and PIER? To understand the origin of all these terms, we must first understand the concept of AR itself.

AR technology was first developed around 50 years ago out of a need to counteract the action of formaldehyde as a fixative.[4] For over a hundred years, formaldehyde-based fixation has served as the standard in surgical pathology, owing to its reliability, availability, and relatively low cost, and so IHC staining methods have needed to be developed with formaldehyde-fixed tissue in mind.[1, 4]

Current research suggests that formaldehyde (and by extension its common derivative, formalin) forms crosslinks between amino acid side chains in tissue proteins.[1] This stabilizes the tissue proteins but as a side effect also masks the epitope binding sites on any antigens of interest, preventing them from being bound by antibodies. Additionally, formaldehyde may alter the hydrogen bonds and electrostatic interactions that mold proteins into their particular configurations, changing the shape of epitope binding sites, which further disrupts any potential antibody-epitope binding.[1]

Before staining, these disruptive actions must be undone, thereby recovering or retrieving the antigen epitopes for binding again. This process has been dubbed antigen retrieval (AR) or epitope retrieval (ER). These two terms are equivalent, but the latter refers specifically to the epitope binding sites on the antigen. The retrieval process has also been referred to as antigen or epitope recovery, unmasking, decloaking, or simply pretreatement.

The first AR methods involved the use of protease enzymes at various times and concentrations to break down the fixation cross-linkages. This method is known as enzymatic antigen retrieval, or enzyme digestion. It also sometimes written as Proteolytic-Induced Epitope Retrieval (PIER) or Protease-Induced Epitope Retrieval (PIER).

However, some antigens are negatively affected by enzymatic digestion and so the introduction of heat-induced AR technology around 20 years later was a major breakthrough.[1, 4] It is hypothesized that heat-induced protein denaturation breaks down the fixative cross-linkages.[1] The use of heated buffer solutions controls for pH, which affects protein configuration and optimizes results. The use of heat to recover antigens is referred to as Heat-Induced Antigen-Retrieval (HIAR) or Heat-Induced Epitope Retrieval (HIER).

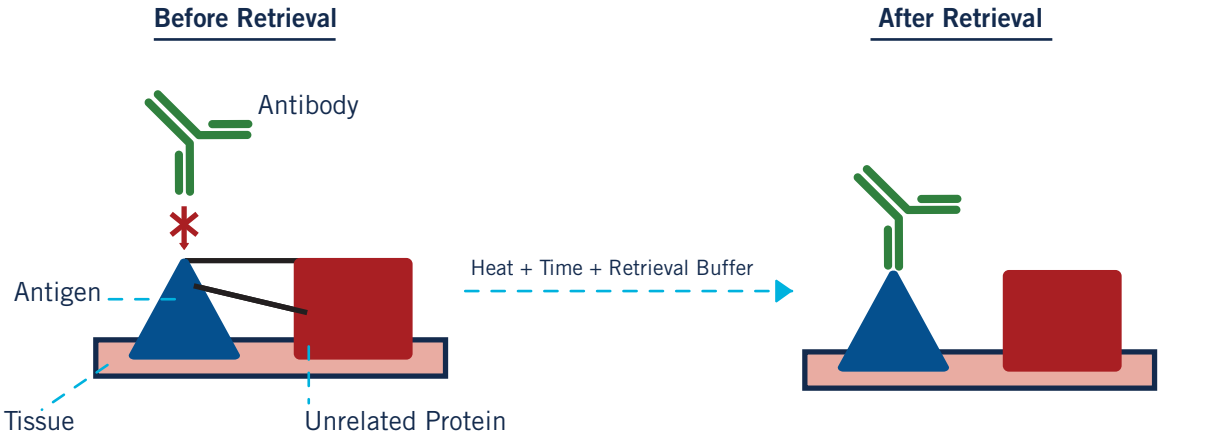
To add to the list of terms, individual IHC companies may also have proprietary names for their AR reagents. Some vendors may only offer one HIER solution while others provide an array of buffer solutions of various pH levels and compositions to fine tune the AR process.

Biocare Medical takes the latter approach and offers a line of HIER buffer solutions referred to as Decloaking Solutions, each with a unique pH and composition to ensure that antigen retrieval for each IHC marker is as optimal as possible. If proteolytic retrieval is required, Biocare also offers several different protease enzymes as part of our Carezyme line.

View our illustration of Heat-Induced Retrieval vs. Enzymatic Retrieval on the following page.

Heat-Induced Retrieval vs. Enzymatic Retrieval

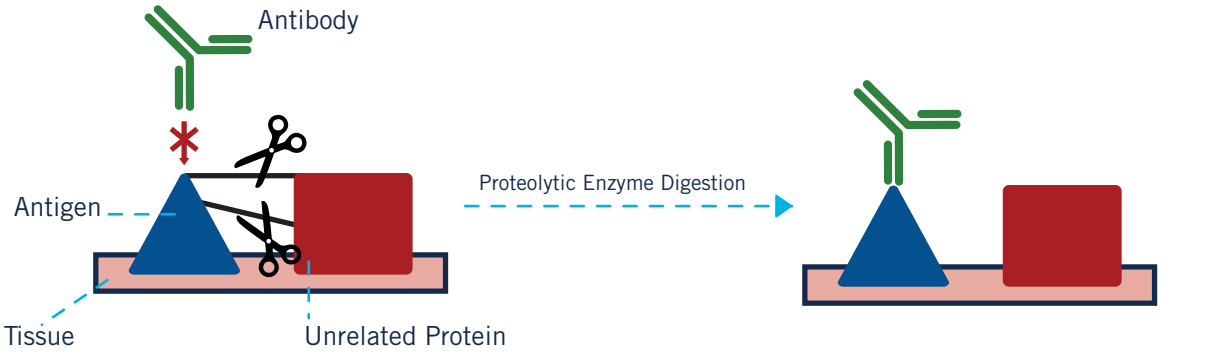
Heat-Induced Retrieval



Crosslinks created by formalin during the fixation process prevent the antibody from binding the antigen of interest

Time spent in heated retrieval buffer breaks the formalin-created crosslinks, allowing the antibody to bind the antigen of interest

Enzymatic Retrieval



Proteolytic enzymes cleave the formalin-created crosslinks

The antibody is able to bind the antigen of interest

Terms for Retrieval Using Enzymes	Terms for Retrieval Using Heat
Enzymatic Antigen Retrieval	
Proteolytic-Induced Epitope Retrieval (PIER)	Heat Induced Epitope Retrieval (HIER)
Protease Induced Epitope Retrieval (PIER)	Heat Induced Antigen Retrieval (HIAR)
Enzymatic Digestion	

To learn more, please visit us at biocare.net or call 1-800-799-9499. Our highly knowledgeable Technical Support staff is available to help you determine which retrieval methods would be best for your lab.

1. Leong, T. Y., & Leong, A. S. (2007). How does antigen retrieval work?. *Advances in anatomic pathology*, 14(2), 129–131. https://doi.org/10.1097/PAP.0b013e31803250c7
2. Shi, S.-R., Cote, R. J., & Taylor, C. R. (1997). Antigen Retrieval Immunohistochemistry: Past, Present, and Future. Journal of Histochemistry & Cytochemistry, 45(3), 327–343. https://doi.org/10.1177/002215549704500301
3. Shi, S.-R., Cote, R. J., & Taylor, C. R. (2001). Antigen Retrieval Techniques: Current Perspectives. Journal of Histochemistry & Cytochemistry, 49(8), 931–937. https://doi.org/10.1177/002215540104900801
4. Shi, S. R., Shi, Y., & Taylor, C. R. (2011). Antigen retrieval immunohistochemistry: review and future prospects in research and diagnosis over two decades. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, 59*(1), 13–32. https://doi.org/10.1369/jhc.2010.957191