

## Cell Marque™ Tissue Diagnostics

**A Combination of HIER and EIER  
Create an Optimal Protocol for Desmoglein 3**

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**Background:**

In the world of pathology, tissues are generally fixed in formalin and embedded in paraffin in order to preserve the sample for diagnostic testing. As a consequence of this procedure, epitopes of the tissue become obscured and need to be uncovered before immunohistochemistry (IHC) testing can take place. The two most common epitope retrieval methods in IHC are heat-induced epitope retrieval (HIER) and enzyme-induced epitope retrieval (EIER). HIER utilizes a heat source (usually a pressure cooker, microwave, steamer, water bath or autoclave) in combination with buffers to reduce cross linkages and restore secondary or tertiary structures of the tissue's epitopes. EIER, on the other hand, utilizes the properties of enzymes such as Proteinase K, trypsin or pepsin to cleave the peptides masking the epitopes of tissues.

In the development of a new desmoglein 3 (DSG3) antibody, it was noted that both heat retrieval and enzyme retrieval individually gave borderline results in unmasking the epitopes for this antigen. It was hypothesized that a combined retrieval protocol would give optimal staining results when compared to the individual retrieval methods. The results are illustrated in this experiment.

**Design:**

Stain three lung squamous cell carcinoma tissues so that the different methods are shown. All manual stains will use DSG3 (EP306) diluted 1:25 with 10 minute antibody incubation.

**Enzyme Only Protocols Tested (EIER ONLY):****DAB Chromogen**

1. Protease (5m) @RT / Peroxide (10m) / DSG3 (10m) / HRP (10m) / DAB (5m) / Hematoxylin
2. Protease (10m) @RT / Peroxide (10m) / DSG3 (10m) / HRP (10m) / DAB (5m) / Hematoxylin
3. Protease (15m) @RT / Peroxide (10m) / DSG3 (10m) / HRP (10m) / DAB (5m) / Hematoxylin

**Permanent Red Chromogen**

4. Protease (5m) @RT / DSG3 (10m) / AP (10m) / PermRed (15m) / Hematoxylin
5. Protease (10m) @RT / DSG3 (10m) / AP (10m) / PermRed (15m) / Hematoxylin
6. Protease (15m) @RT / DSG3 (10m) / AP (10m) / PermRed (15m) / Hematoxylin

**Heat Retrieval Only Protocols Tested (HIER ONLY):****DAB Chromogen**

7. Pressure cooker high pH EDTA (15m) / Peroxide (10m) / DSG3 (10m) / HRP (10m) / DAB (5m) / Hematoxylin

**Permanent Red Chromogen**

8. Pressure cooker high pH EDTA (15m) / DSG3 (10m) / AP (10m) / PermRed (15m) / Hematoxylin

