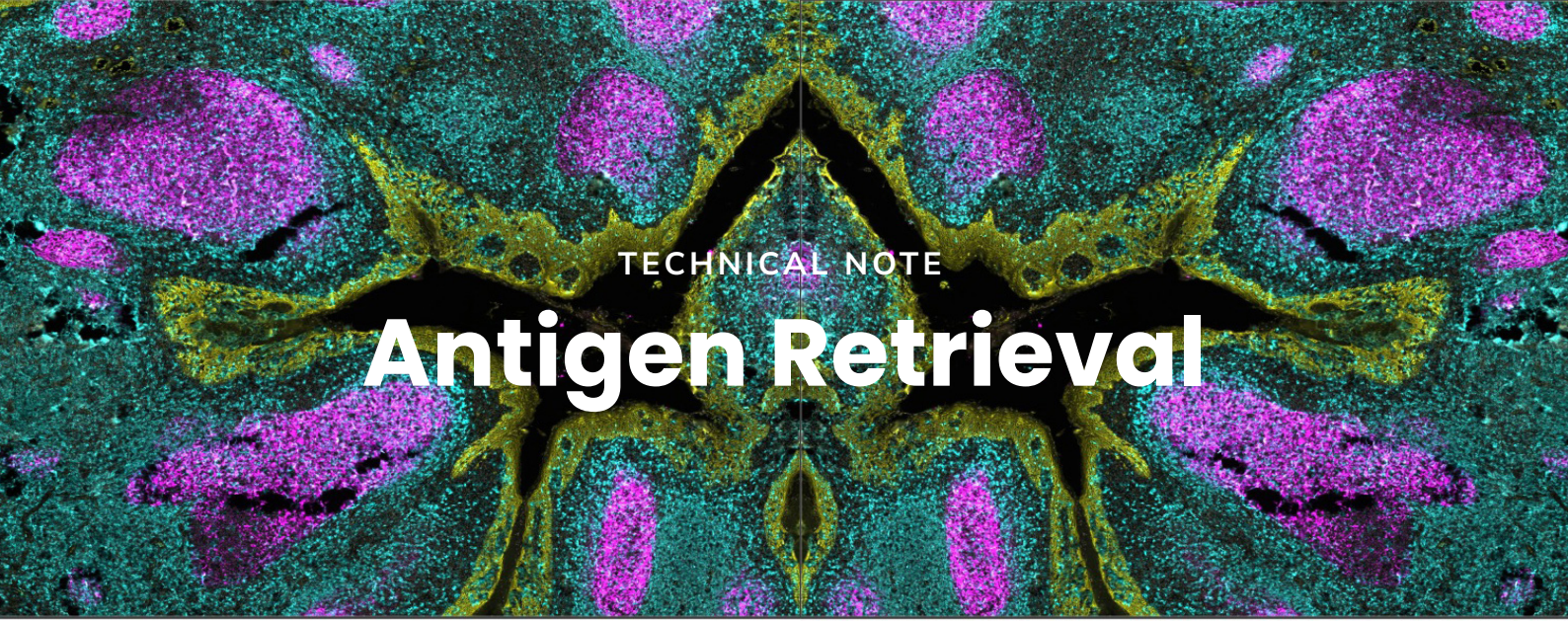


TECHNICAL NOTE

Antigen Retrieval

Antigen retrieval is a critical sample preparation step in immunohistochemistry and related techniques such as *in situ* proximity ligation assay (*is*PLA). During formalin fixation of tissue samples, methylene bridges are formed between proteins, cross-linking them and masking the antigenic sites (epitopes) that antibodies need to recognize. Without retrieval, many antibodies will fail to bind their target, resulting in weak or absent staining.

There are two principal approaches to antigen retrieval:

1. Heat-Induced Epitope Retrieval (HIER):
The tissue section is immersed in a retrieval buffer solution and subjected to controlled heating. The combination of heat and buffer chemistry breaks the methylene cross-links formed during fixation, restoring the three-dimensional conformation of the epitopes. HIER is the most widely used retrieval method and is effective for the majority of antigens.

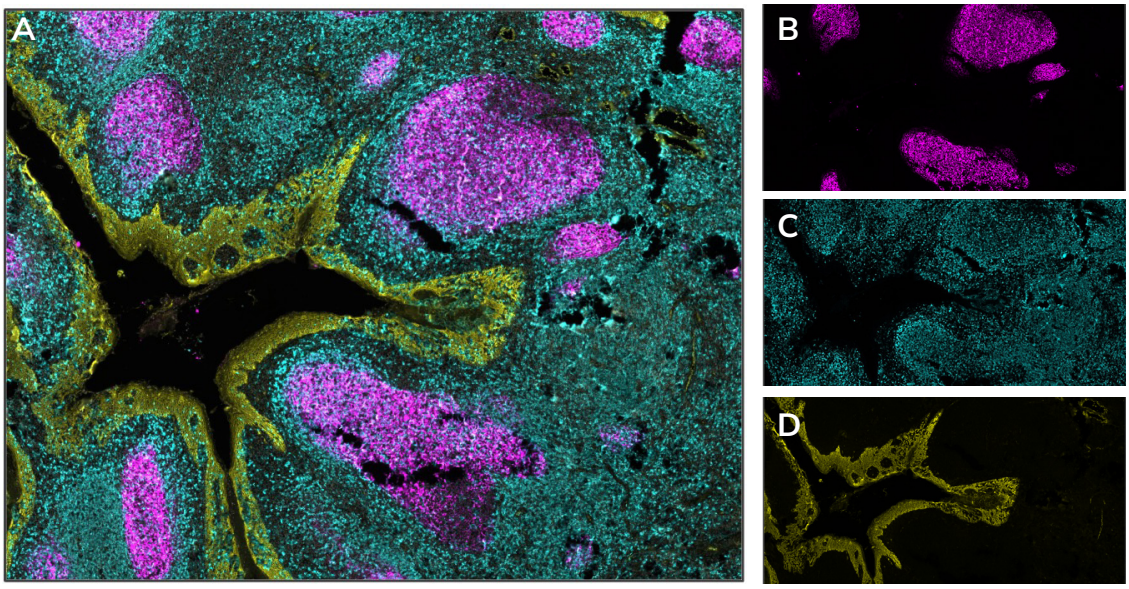
2. Proteolytic-Induced Epitope Retrieval (PIER):
Enzymatic digestion with proteases such as trypsin, proteinase K, or pepsin is used to cleave cross-linked proteins and expose epitopes. PIER may be preferred for certain antigens, particularly extracellular matrix proteins, but carries a risk of damaging tissue morphology if over-digested.

The Navinci Antigen Retrieval Buffer pH 9.0 (10X) is intended for use with the HIER method. The buffer operates at a high pH (9.0), which is particularly effective for a broad range of epitopes. The Tris-EDTA formulation at high pH has been shown to provide superior retrieval for many targets compared to lower-pH citrate-based buffers, especially for nuclear and membrane-associated antigens.
For HIER, controlled and reproducible heating is essential. The use of a pressure cooker or a dedicated antigen retrieval chamber is recommended to ensure stable temperature control throughout the procedure. Uncontrolled heating (e.g. open water baths or microwaves) may lead to variable results due to uneven temperature distribution and evaporation.

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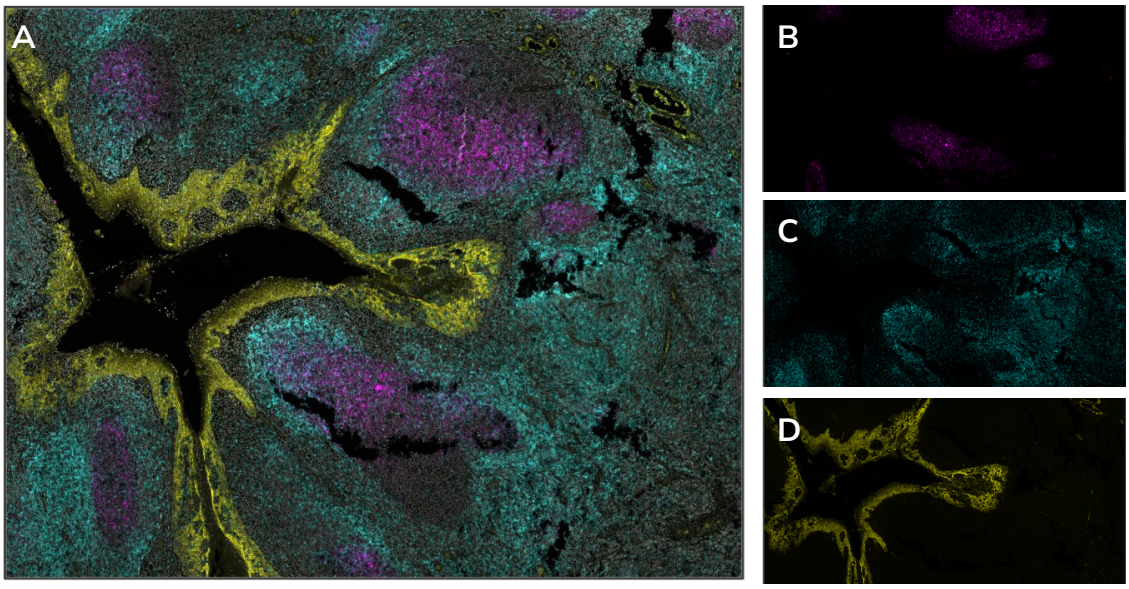
Demonstration of Navinci Antigen Retrieval Buffer on Omni, 9-plex *is*PLA assay

Figure 1:
Navinci antigen retrieval buffer and protocol, pH 9.0



A) Merged image, B) CD21 in purple, C) CD4 in teal, D) TROP2 in yellow

Figure 2:
Competitor antigen retrieval buffer and protocol, pH 9.0



A) Merged image, B) CD21 in purple, C) CD4 in teal, D) TROP2 in yellow

Navinci's Antigen Retrieval Buffer is designed to effectively unmask these epitopes, restoring accessibility and enabling optimal binding of *is*PLA probes. The result is improved signal generation, enhanced sensitivity, and more consistent assay performance.

For more information or to place an order, visit navinci.se/products or contact us.

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