Antigen Retrieval: Protease-mediated (PIER) or Heat-mediated (HIER)

Formaldehyde / Formalin is the most common fixative in diagnostic pathology because it preserves tissue morphology very well. However, most formalin-fixed tissues require an antigen retrieval step to unmask antibody binding sites. Antigen retrieval techniques use either heat (Heat Induced Epitope Retrieval = HIER) or enzymes (Proteolytic Induced Epitope Retrieval = PIER).

Widely used enzymes in the PIER method include Proteinase K, Trypsin, and Pepsin and are usually incubated for 10 – 30 min at 37°C. However, enzymes may have a destructive effect on tissue morphology and the success rate for restoring immunoreactivity is low.

The SYSY standard protocol consists of a HIER step using a food steamer, which enables consistent and standardized antigen retrieval conditions. Heat-induced antigen retrieval can also be performed using a microwave, a pressure cooker, or a water bath set to 95°C. Antigen retrieval in easily damaged tissues can be done overnight in a water bath set to 60°C.

HIER step using a food steamer:

Heat the steamer with a suitable staining container filled with antigen retrieval buffer until the temperature reaches 97°C. Transfer sections into the staining container, wait until the temperature reaches 97°C again, and then start the antigen retrieval time. When the antigen retrieval time has elapsed, remove the staining container and allow the buffer to cool down for 20 min (target end temp. ~60°C). Continue with the immunohistochemical staining protocol.

SYSY uses the following antigen retrieval buffers, which reflect the most popular buffers for HIER:

- Citrate Buffer (10 mM Citrate, 0.05% Tween 20, pH 6.0)
- Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA, 0.05% Tween 20, pH 9.0) *
- EDTA buffer (1 mM EDTA, 0.05% Tween, pH 8.0) *

** This buffer works excellently for most antibodies and can highly improve sensitivity, but often results in high background*

HIER is extremely sensitive to pH, time, temperature, and buffer composition. Generally, the higher the temperature, the shorter the heating time and vice versa. Specially formulated commercially available antigen retrieval buffers may be superior to SYSY buffers.

There is no universal method for antigen retrieval and no single HIER solution that is optimal for all antigens. Therefore, we recommend optimization of HIER using different time and pH / buffer conditions empirically for each antibody and each tissue type.