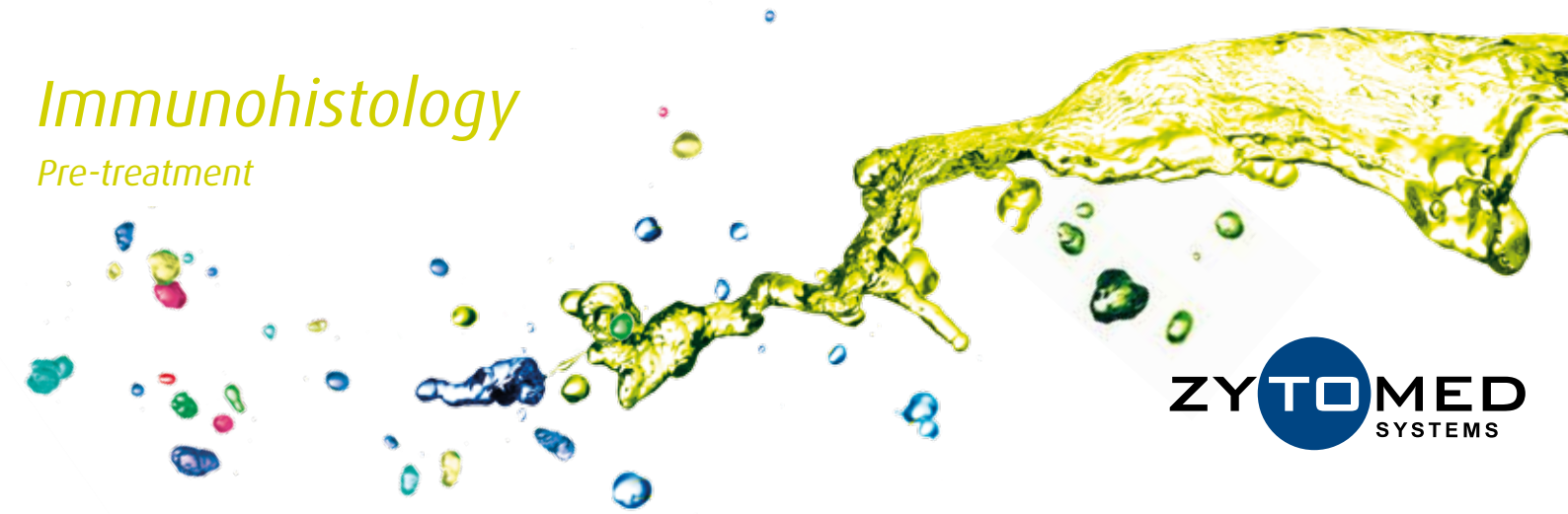
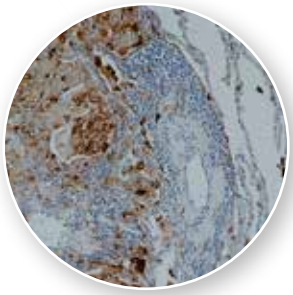
Immunohistology

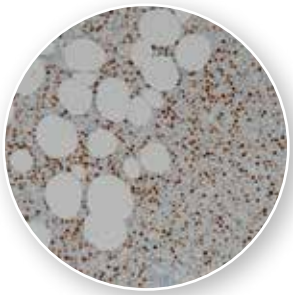
Pre-treatment



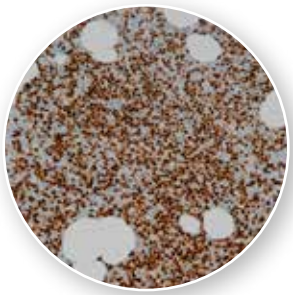
ZYTOMED SYSTEMS



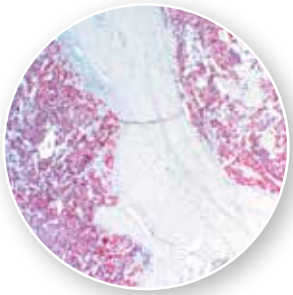
MAdL (MSK083-05) staining on adeno carcinoma of the lung, pre-treatment with Pepsin (ZUC038)



Progesterone Receptor (RBK020), pre-treatment with Citrate Buffer pH 6.0 (ZUC028)



Progesterone Receptor (RBK020), pre-treatment with T-EDTA Buffer pH 9.0 (ZUC029)



TTF-1 (MSK044) staining on lung carcinoma, pre-treatment to harsh, tissue partly floated off

Pre-treatment for formalin-fixed paraffin-embedded tissue sections

For optimal staining results in immunohistochemistry primary antibodies must be able to bind to their target antigen. If this is not guaranteed false-negative staining results might be obtained even if the correct primary antibody and detection system were used.

Nowadays, buffered formalin is the most widely used fixative for tissue sections. There are many advantages of this fixative. It is cheap, easy to prepare, and compatible with most of the commonly used histochemical stains. It stabilises the cell structure via cross-linking of proteins and reactive groups.

For immunohistochemistry this cross-linking is a big disadvantage because the modified protein structure makes it impossible for almost all antibodies to bind to their epitope (the specific area of the protein were the antibody binds). This effect is referred to as "antigen masking".

Protease digestion was the first method used to counteract the antigen masking effects of formalin. An immunohistochemical revolution was the development of heat induced epitope retrieval (HIER) by Dr. Shi and colleagues. HIER as well as protease digestion is able to partially restore the original protein configuration, "unmask" the epitopes and make them accessible for primary antibodies.

In HIER, de-paraffinsed tissue sections are placed in a buffer solution and treated with heat. The tissue sections are cooked in the buffer solution for a certain period of time. Initially microwave ovens were used as a source of heat but this technique has recently been replaced by other techniques using steamer or pressure cooker as heat source. Both techniques are milder for the tissue sections, lead to better morphology and are easier to standardize.

Unfortunately, there is no single HIER solution that is best for all antigens. The pH of the buffer solution used can affect the final results. Citrate buffer of pH 6.0 (Zytomed Systems Citrate Buffer pH 6.0 ZUC028) is the most common pre-treatment buffer used in HIER. However, a better staining intensity can be obtained for many antibodies by using buffers of higher pH value. Here, EDTA buffer of pH 8.0 or Tris-EDTA buffer of pH 9.0 could by a good choice (Zytomed Systems EDTA Buffer pH 8.0 ZUC040 or Tris-EDTA Buffer pH 9.0 ZUC029).

Some critical points have to be noticed when using HIER.

Tissue sections should always be fixed in 4% buffered formaldehyde for 6–24 hours. Fixation time depends on the tissue size. Tissue fixation in un-buffered formaldehyde or overfixation may require longer pre-treatment times.

Sometimes tissue sections tend to float off during HIER. This can have different reasons. It is most important to always use adhesive slides when immunohistochemical staining is planned. Especially thick sections or sections containing fatty tissue often tend to float off. For these tissues it can be an improvement to cut thinner sections and to dry the tissue sections for a longer time after placing the sections on the slides. In general drying overnight at 50–60°C is optimal for all IHC slides.

Let's end with a statement of Rodney T. Miller, MD, Director of Immunohistochemistry in the ProPath Laboratory in Dallas, Texas (USA) on the 2001 Annual Meeting of the Society of Applied Immunohistochemistry: *"Quality in IHC starts at the grossing table, and it is important that sections for processing be cut thin (ideally 2 µm in thickness) and not crammed into processing cassettes. If good H&E sections cannot be obtained from processed material, the immunostains will also be suboptimal, and there is much truth to the saying „GARBAGE IN – GARBAGE OUT".*

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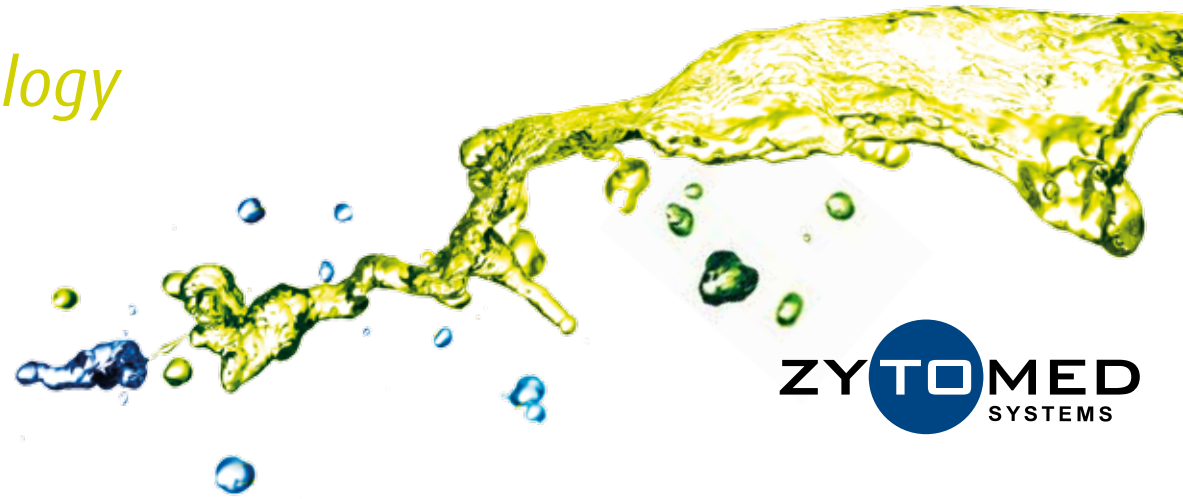

Procedure I: Pressure cooker

1. Fill pressure cooker with demineralised or distilled water.
   You need ca. 1.5 litre water for most pressure cookers.
2. Fill Coplin jar(s) with prepared HIER solution. Make sure there is enough solution to cover the tissue sections on the slides completely. Place Coplin jar(s) with HIER solution in the pressure cooker and place the lid loosely on the cooker.
3. Heat pressure cooker with lid in place but not closed on a heating plate until the pre-treatment solution reaches 95°C.
4. Place slides with tissue sections into the preheated solution. Cover the Coplin jars loosely with lids. Tissue sections must be completely covered with HIER solution.
5. Close the lid of the pressure cooker and heat until maximum pressure is reached.
   Usually two rings of the pressure gauge are visible.
6. Leave the pressure cooker for additional 10 minutes on the heating plate.
7. Switch off heating plate. Place the pressure cooker under running tap water until it is cooled down and the excess pressure is relieved.
8. Open the now non-pressurised cooker and let cold tap water run in until all water inside as well as the pre-treatment solution in the jars is replaced by tap water.
9. Transfer tissue slides into Wash Buffer and start with immunohistochemical staining.

Procedure II: Steamer

1. Fill steamer up to the mark “maximum“ with distilled water and heat with lid closed until the water is boiling (ca. 10 minutes).
2. Fill Coplin jar(s) with prepared HIER solution. Please make sure there is enough solution to cover the tissue sections on the slides completely. Place Coplin jar(s) with HIER solution in the steamer and place the lid(s) loosely on Coplin jar(s).
3. Close steamer and heat for 30 minutes to preheat the pre-treatment solution inside the Coplin jar(s).
4. Place slides with tissue sections into the preheated solution. Cover the Coplin jars loosely with lids. Tissue sections must be completely covered with HIER solution.
5. Close steamer and heat again for 30 minutes.
6. Switch off steamer, remove Coplin jar(s) and let them cool down for 20 minutes on the laboratory bench.
7. Let tap water run slowly into the jar(s) until all pre-treatment solution is replaced by tap water.
8. Transfer tissue slides into Wash Buffer and start with immunohistochemical staining.

Anhaltinerstraße 16 | 14163 Berlin | Germany | Fon +49 30 804 984 990 | Fax +49 30 804 984 999 | international@zytomed-systems.de | www.zytomed-systems.com