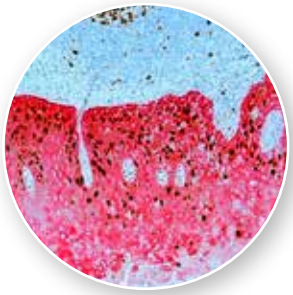
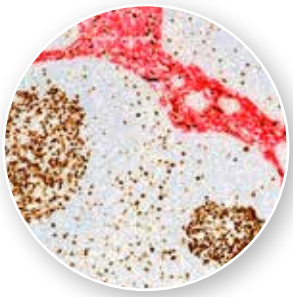
Immunohistology

Double stain H_20 elution technique

ZYTOMED SYSTEMS



Ki67 (DAB) + CK pan
(Permanent AP Red) staining on tonsil



Ki67 (DAB) + CK pan
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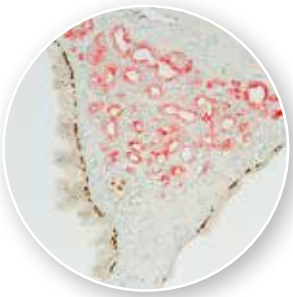
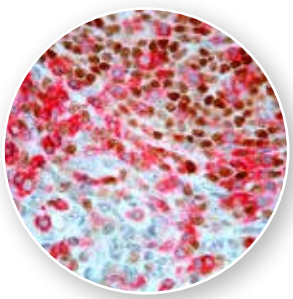


p63 (DAB) + P504S (Permanent AP Red)
staining on prostate cancer



Pax-5 (DAB) + CD3
(Permanent AP Red) staining on tonsil

Double stain protocol for immuno-histochemistry – H20 elution technique

Immunohistochemical double stains are easily performed by using Zytomed Systems Double Stain Polymer Detection Kit. However, this is only possible if one primary antibody is from mouse, the other one from rabbit.
When the two primary antibodies are obtained from the same species another technique has to be used. For this purpose a simple procedure using an elution with hot deionised water is carried out.

Some remarks for this protocol:

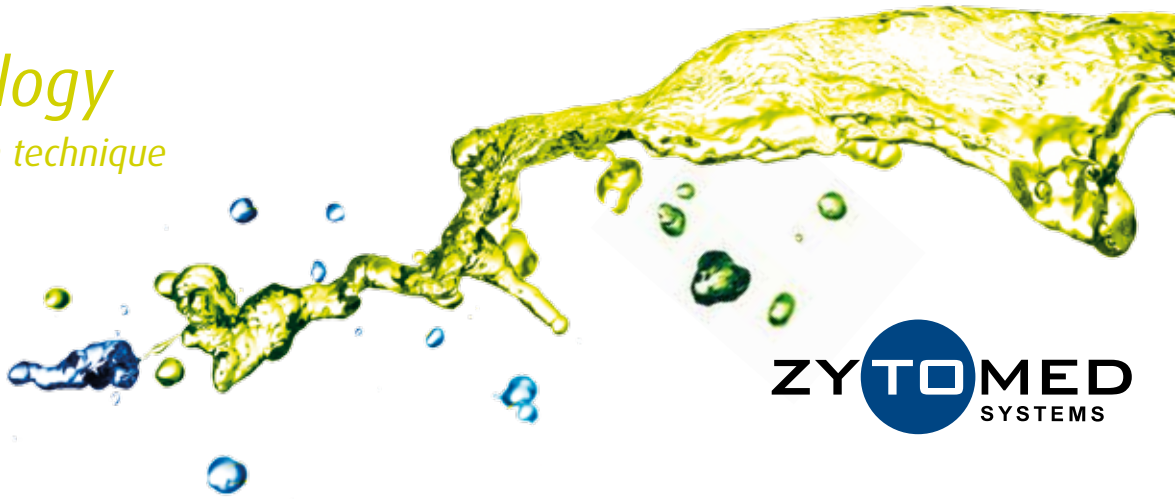
- Both primary antibodies should need the same pre-treatment.
- The first-used chromogen has to be stable in hot deionised water. Usually, we use DAB (DAB057, DAB530) for this first chromogenic step, but other chromogenic substrates like Permanent AP Red (ZUC001) or AEC Single Solution (ZUC037) can also be used.
- Both primary antibodies should have different target cell structures (i.e. one primary antibody stains the cytoplasm, the other one the nucleus).
- Do not apply both primary antibodies in a mixture on the slide.
- The elution step can also be carried out in a water bath or microwave oven. The exact conditions have to be determined by the user.

Protocol for IHC double staining – H_20 elution technique

- Deparaffinise and rehydrate paraffin-embedded tissue sections
- Apply and incubate Peroxide Block (3 % H_2O_2 solution) for 10 min
- Rinse with tap water
- Pre-treatment with HIER (Heat Induced Epitope Retrieval) or enzymatic digestion
- Rinse with Wash Buffer
- Optional: apply and incubate protein block for 5 min. Let protein block drain away
- Apply and incubate first primary antibody for 60 min
- Apply HRP One-Step Polymer anti-Mouse/Rabbit/Rat for 30 min
- Rinse with Wash Buffer
- Apply and incubate DAB working solution for 10 min
- Rinse with deionised water
- Elution in hot deionised water in a steamer for 10 min (see protocol below)
- Rinse immediately in tap water and afterwards in Wash Buffer
- Optional: apply and incubate protein block for 5 min. Let protein block drain away
- Apply and incubate second primary antibody for 60 min
- Rinse with Wash Buffer
- Apply and incubate AP Polymer anti -Mouse/Rabbit for 30 min.
- Rinse with Wash Buffer
- Apply and incubate Permanent AP-Red working solution for 20 min
- Rinse with tap water
- Counterstain with haematoxylin
- Blueing with tap water
- Dehydrate through a graded series of ethanol and clear in xylene
  Mount with a permanent mounting medium

Immunohistology

Double stain H_20 elution technique



H_20 elution in a steamer

- Fill steamer up to the mark "maximum" with distilled water and heat with lid closed until the water is boiling (ca. 10 minutes).
- Fill Coplin jar(s) with deionised water. Please make sure there is enough water to cover the tissue sections on the slides completely. Place Coplin jar(s) with water in the steamer and place the lid(s) loosely on Coplin jar(s).
- Close steamer and heat for 30 minutes to preheat the deionised water inside the Coplin jar(s).
- Place slides with tissue sections into the preheated water. Cover the Coplin jar(s) loosely with lid(s). Tissue sections must be completely covered with water.
- Close steamer and heat again for 10 minutes.
- Switch off steamer, remove Coplin jar(s) and let cold tap water run slowly into the jar(s) until all hot deionised water is replaced by tap water.
- Transfer tissue slides into Wash Buffer and proceed with immunohistochemical staining.

Reagents used

- Peroxide Block ZUC019-100
- Citrate Buffer pH6.0 ZUC028-500 (for HIER)
- Wash Buffer ZUC020-500
- ZytoChem Plus HRP One-Step Polymer anti-Mouse/Rabbit/Rat ZUC053-006
- DAB Substrate Kit DAB530
- ZytoChem Plus (AP) Polymer Kit anti-Mouse/Rabbit POLAP-006
- Permanent AP Red ZUC001-125

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