Antigen Retrieval for Immunohistochemistry with Paraffin-Embedded Tissues

Maria Beatriz Padua[1] and Peter J. Hansen[1]

[1]*Dept. of Animal Sciences, University of Florida*

Background

Tissue sectioning with paraffin embedded tissues offers several advantages as compared to work with frozen tissues including ease and the fact that microtomes are generally more widely available than cryostat microtomes. Unfortunately, many antigens do not survive the fixation and embedding process because of covalent and non-covalent modifications to the protein. In some cases, antigen retrieval techniques allow antigenicity to be re-established. The following technique is one that we have used to for immunohistochemistry on paraffin-embedded sections. It is based on use of microwave energy to effect antigen retrieval. The protocol itself was adapted from one obtained by Upstate. The immunohistochemistry procedure, adapted from Biomeda's manual, is for use of **Biomeda's Histo Scan** kit based on a streptavidin-peroxidase/biotinylated second antibody detection system with 3-amino, 9-ethylcarbazole (AEC) as chromogen. Undoubtedly, other kits or home-made reagents will also work but we have found Biomeda's technology to be effective for a variety of antigen-antibody systems.

Buffers and Solutions

- Protocol Safe Clear – xylene substitute (Fisher).
- 100% ethanol, 95% ethanol and 80% ethanol. Prepare the 80% ethanol fresh each day.
- Phosphate buffered saline (PBS) pH 7.4
- Oxidation blocking solution (PBS + 2% hydrogen peroxide). Make fresh each day.
- Antigen retrieval solution (10 mM citrate buffer, pH 6.0).
- Automation buffer [PBS + 2% (v/v) goat serum]. Made fresh and keep at 4°C.
- *Note: do not add azide to buffers since it inhibits peroxidase activity*

Procedure for Antigen Retrieval

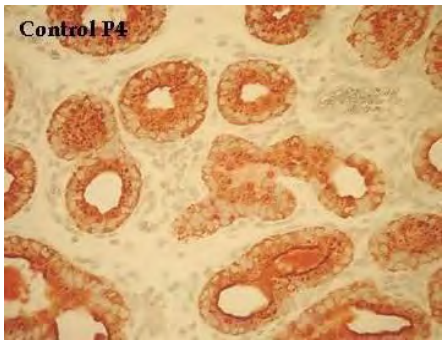
1. Label all slides using a pencil.
2. In the hood, place 8 coplin jars in a row. Place the slides in the removable rack. Deparaffinize and rehydrate as follows:
   - Three times for 5 minutes in Protocol Safe Clear
   - Two times for 5 minutes in 100% ethanol
   - Two times for 5 minutes in 95% ethanol
   - Once for 5 minutes in 80% ethanol
   - Approximately, 150 ml of the solutions are enough to cover the removable rack for 10 to 20 slides, 70 x 85 mm coplin jar. Solutions should be clean each time.
3. Outside the hood, prepare 5 glass staining dishes for the next steps (75 x 25 mm, for 16 slides, holds slides vertically). Approximately 55 – 60 ml of the solutions are enough to cover the slides.
4. Immediately after removal from 80% ethanol solution, place the slides into a glass staining dish containing the antigen retrieval solution. Place this glass staining dish into a beaker filled with water and microwave on high for three minutes (700 watt oven).
5. Check level of the retrieval solution; allow cooling for 3 minutes at room temperature. Repeat this step 3 – 4 times.
6. Remove the glass staining dish from the beaker and cool for 20 minutes at room temperature.
7. Rinse slides in deionized water, two times for 5 minutes.
8. Place the slides in oxidation blocking solution once for 5 minutes
9. Rinse the slides in PBS once for five minutes. After this step, keep the slides in a humidified chamber until staining procedure.

Procedure for Immunohistochemistry

1. Blot the area around the sample to remove excess buffer. Use a Pap-Pen to surround the area around the sample.
2. Incubate the slides with 2 drops of tissue conditioner (from kit) for 5 minutes at room temperature. Rinse the slides with automation buffer for 3 minutes. Using a kimwipe, remove the excess buffer from the slides and quickly proceed to next step. DO NOT LET SLIDES DRY.
3. Incubate slides with enough drops (~4) of the primary antibody using the recommended dilution in automation buffer. Incubate negative control sections with the appropriate control (purified mouse IgG, control ascites fluid or normal rabbit serum) instead of antibody.
4. Incubations can be performed for a variable time but typically 30 minutes at room temperature is sufficient. Afterwards, rinse the slides with automation buffer for 3 minutes. Remove excess buffer.
5. Incubate positive and negative controls with 4 drops of the secondary antibody (provided in kit) for 30 minutes at room temperature. Note that the Histoscan kit is for monoclonal antibodies - other kits are available for polyclonal antibodies made in species other than the mouse.
6. Rinse the slides with automation buffer for 3 minutes. Remove excess buffer.
7. Incubate the slides with 4 drops of Peroxidase reagent (from the kit) for 30 minutes at room temperature. Rinse the slides with automation buffer for 3 minutes. Remove excess buffer.
8. Incubate the slides for 15 minutes at room temperature with 4 drops of the working reagent from ingredients included in the kit as follows:
    - 5 ml of double distilled water
    - 2 drops of buffer (solution 3A)
    - 1 drop of chromogen (solution 3B)
    - 1 drop of substrate (solution 3C)

    Note: this solution should be made fresh and used within 4 hours.
9. Rinse the slides with double distilled water for 3 minutes. Remove the excess water and quickly proceed to next step.
10. Incubate the slides with 4 drops of hematoxylin (from kit) for 3 minutes at room temperature. Rinse the slides with ddWATER for 3 minutes. Rinse the slides again in running tap water, until hematoxylin in the tissue turns blue.
11. Remove the excess water from the slides and add 1 drop of Crystal/Mount (BioMeda Cat No. M02) or other mounting medium to each sample. Cover the samples with glass cover slips.

Example of Results



Localization of ovine uterine serpin in the endometrium of a progesterone-treated, ovariectomized ewe. Sections were incubated with a monoclonal antibody to uterine serpin followed by detection using the Biomeda HistoScan kit.

Modified 09-30-2003