ROCKLAND

Antigen Retrieval Methods

Formalin fixed tissue requires an antigen retrieval step before immunohistochemical staining can proceed. This is due to the formation of methylene bridges during fixation, which cross link proteins and therefore mask antigenic sites. The two methods of antigen retrieval are either enzymatic or heat mediated. Both serve to break the methylene bridges and expose the antigenic sites in order to allow the antibodies to bind. Some antigens prefer the enzymatic method to heat mediated antigen retrieval and vice versa. The enzymatic method tends to be a gentler process than heat mediated method, so is best suited to more sensitive tissues. However, the enzymatic method tends to take much longer and is more technically demanding.

If no antigen retrieval step is stated on the antibody data sheet, start off by trying the heat mediated method. If at first you don't succeed, try again using the enzymatic method.

Frozen tissue sections do not need an antigen retrieval step. Once mounted on APES coated slides, they are best kept at -80°C until needed. When required, allow the slides to warm at room temperature for 5 minutes, then acetone fix for 5 minutes followed by a PBS or TBS rinse. Afterwards, continue with the immunohistochemical staining protocol.

Enzymatic Antigen Retrieval Method

Tissue sections are best mounted on APES (amino-propyl-tri-ethoxy-silane) coated slides. Slides should be placed in a standard rack for this procedure.

I. Reagents Required

Reagent	Preparation
Alpha-Chymotrypsin (type II from Bovine pancreas) 0.1 g	N/A
Calcium Chloride 0.1 g	N/A
UltraPure Sterile Water (MB-009-1000)	N/A
0.1 N Sodium Hydroxide Solution (for pH adjustment	N/A
0.1 N Hydrochloric Acid Solution (for pH adjustment)	N/A
Xylene	N/A
Methanol Industrial Methylated Spirits (IMS) or Methanol	N/A

II. Procedure

1. Set a water bath to read 37°C. Add the required amount of UltraPure Water into each trough and then place the troughs into the water bath. Allow the UltraPure Water to warm to 37°C.
2. Dewax and re-hydrate paraffin sections by placing them in 3 changes of xylene for 3 minutes each, followed by 3 changes of IMS or methanol for 3 minutes each, followed by cold running tap water for 3 minutes.
   Note: At no time from this point onwards should the slides be allowed to dry out.
3. Place slides in one trough of UltraPure Water at 37°C to warm.
4. Remove the other trough and into this dissolve the calcium chloride and freshly prepared chymotrypsin using a magnetic stirrer. Once dissolved, pH to 7.8 using the sodium hydroxide and hydrochloric acid solutions. Return the trough to the water bath and allow this enzyme solution to re-heat to 37°C.
5. Transfer the warmed slides into the enzyme solution for a suggested 20 minutes then remove the slides and place them into cold running tap water for 3 minutes.
6. Continue with immunohistochemical staining protocol.

Microwave Method

The use of a domestic microwave is inadvisable. Hot and cold spots are common, which lead to uneven antigen retrieval. In addition, antigen retrieval times are usually longer, due to the absence of a pressurized environment that nearly always leads to section dissociation.

A scientific microwave is much more appropriate. Most brands have on-board pressurized vessels and can keep the temperature at a constant 98°C to avoid section dissociation. The only drawback is the expense of purchasing one! But if your department has one, use it.

Tissue sections are best mounted on APES (amino-propyl-tri-ethoxy-silane) coated slides. Slides should be placed in a standard plastic rack for this procedure.

I. Reagents Required

Reagent	Preparation

Tri-Sodium Citrate 2.94 g	N/A
0.2 M Hydrochloric Acid Solution 22.0 mL	N/A
UltraPure Sterile Water (MB-009-1000)	N/A
0.1 N Sodium Hydroxide Solution (for pH adjustment)	N/A
0.1 N Hydrochloric Acid Solution (for pH adjustment)	N/A
Xylene	N/A
Methanol Industrial Methylated Spirits (IMS) or Methanol	N/A

II. Procedure

1. De-wax and re-hydrate the paraffin sections by placing them in 3 changes of xylene for 3 minutes each, followed by 3 changes of IMS or methanol for 3 minutes each, followed by cold running tap water. Keep them in the tap water until the microwave antigen retrieval solution has been prepared. At no time from this point onward should the slides be allowed to dry out!
2. Add the tri-sodium citrate, hydrochloric acid and UltraPure Water together in a 1 L beaker/conical flask. Use a magnetic stirrer to ensure that all reagents are properly dissolved. Adjust to pH 6.0 using the sodium hydroxide and hydrochloric acid solutions. Add this solution to the microwaveable vessel.
3. Remove the slides from the tap water and place them in the microwaveable vessel. Place the vessel inside the microwave. If domestic, set to full power and wait until the solution comes to the boil. Boil for 15 minutes from this point. If scientific, program so that antigens are retrieved for 15 minutes once the temperature has reached 98°C.
4. When 15 minutes has elapsed, remove the vessel and run cold tap water into it for 10 minutes.
5. Continue with immunohistochemical staining protocol.