



## Antigen Retrieval Protocol for IHC

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 main methods of antigen retrieval are Enzymatic and Heat Mediated. Both methods serve to break the methylene bridges and so 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 much 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 that method fails, try again using the enzymatic method.

Frozen tissue sections do not need an antigen retrieval step. Once mounted on APES (amino-propyl-tri-ethoxy-silane) coated slides, they are best kept at -80 deg 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 coated slides. Slides should be placed in a standard rack for this procedure.

#### Reagents Required:

- 100 ug Alpha-Chymotrypsin (type II from Bovine pancreas)
- 100 ug Calcium Chloride
- Ultrapure water
- 0.1 N Sodium Hydroxide solution (for pH adjustment)
- 0.1 N Hydrochloric Acid solution (for pH adjustment)
- Xylene
- Methanol Industrial methylated spirits (IMS) or methanol.

## Procedure:

1. To a 37 deg C water bath, add the required amount of ultrapure water into each trough and then place the troughs in the water bath. Allow the Ultrapure water to warm to 37 deg C.
2. Dewax and rehydrate the paraffin sections by placing them in xylene 3 times for 3 minutes each time, followed by placing them in IMS or methanol 3 times for 3 minutes each time, followed by a rinse in cold running tap water for 3 minutes.
3. At no time from this point onwards should the slides be allowed to dry out!  
Place slides in ultrapure water at 37 degrees C to warm.
4. In a waterbath trough, dissolve the calcium chloride and freshly prepared chymotrypsin using a magnetic stirrer. Once dissolved, adjust 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 deg C.
5. Transfer the warmed slides into the enzyme solution for approx 20 minutes then remove the slides and place them into cold running tap water for 3 minutes.  
Continue with the 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.

While possible to perform this procedure using a domestic microwave, a scientific microwave is far superior. Most brands have on-board pressurized vessels and can keep the temperature at a constant 98 degrees C to avoid section dissociation.

Tissue sections are best mounted on APES coated slides. Slides should be placed in a standard plastic rack for this procedure.

## Reagents Required:

- 2.94 grams of Tri-Sodium Citrate
- 22.0 ml of 0.2 M Hydrochloric acid solution
- Ultrapure water
- 0.1 N Sodium Hydroxide solution (for pH adjustment)
- 0.1 N Hydrochloric Acid solution (for pH adjustment)
- Xylene
- Methanol Industrial methylated spirits (IMS) or methanol.

## **Procedure:**

1. Dewax and rehydrate the paraffin sections by placing them in xylene 3 times for 3 minutes each time, followed by placing them in IMS or methanol 3 times for 3 minutes each time, followed by a rinse in cold running tap water for 3 minutes.

At no time from this point onwards should the slides be allowed to dry out!

2. Add the tri-sodium citrate, hydrochloric acid and ultrapure water together in a 1-liter 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 a microwaveable vessel.
3. Remove the slides from the tap water and place them in the microwaveable vessel. Place the vessel in 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 the antigens are retrieved for 15 minutes once the temperature has reached 98 deg C.
4. After 15 minutes has elapsed, remove the vessel and rinse with cold tap water for 10 minutes.
5. Continue with the immunohistochemical staining protocol.