# Antigen Retrieval Protocol

Formalin fixed paraffin embedded tissue is a very common preparation for a wide variety of tissue types used in immunohistochemistry. Unfortunately, this process can result in the cross-linking of proteins which can mask binding sites on the antigen of interest. In order to unmask, or retrieve, these cross-linked sites, scientists can utilize a variety of procedures known collectively as antigen retrieval. Two of the more common antigen retrieval procedures are heat-induced antigen retrieval (HIER) and enzyme-induced antigen retrieval (EIAR).

There are several heating methods commonly employed in HIER, including steamers, microwave ovens, pressure cookers, and hot water baths. Likewise, there are several enzymes that can be utilized in EIAR, including Proteinase K, Trypsin, and Pepsin. Each antigen retrieval method must be adapted according to the particular antigen, antibody, and tissue type under investigation. Occasionally, certain antigens utilize a combination of retrieval methods. Use caution when choosing a retrieval method. If an enzyme or a heating method is too harsh this can cause the tissue to lift off the slide (such as skin sections), or cause the tissue to become over-digested and/or over-retrieved.

### Enzyme-Induced Antigen Retrieval (EIAR):

Proteinase K is a common EIAR enzyme, which can be purchased as a ready-to-use reagent from a supplier. Follow the manufacturer's instructions.

#### Proteinase K Antigen Retrieval:

1. Prepare the tissue section as required for the selected immunostaining protocol.
2. Wash the tissue section with DI twice for three minutes each.
3. Incubate the tissue section with Proteinase K for 10 minutes. Monitor the sample; the enzyme can cause the tissue to lift off the slide.
4. Rinse the tissue section with DI and wash the slide in DI three times for 1-2 minutes each.
5. Wash the slide in wash buffer twice for three minutes each.
6. Continue with the selected immunostaining protocol.

### Heat-Induced Antigen Retrieval (HIER):

10mM Sodium Citrate, pH 6.0 retrieval buffer is a common HIER buffer. This buffer can be purchased as a ready-to-use reagent or prepared in the lab by the researcher. Other buffers include Tris pH 8.0 and Tris/EDTA pH 9.0. The optimal pH and retrieval solution should be determined for each tissue type as well as the appropriate incubation times.

#### 10mM Sodium Citrate, pH 6.0:

1. Prepare 1.0 L stock solution of each of the following. Measure the reagent and fill the beaker to 1.0 L with DI (Filter the solutions).
   0.1M sodium citrate: 29.41 g sodium citrate dihydrate
   (FW = 294.10 g/mol)

   0.1M citric acid: 21.01 g citric acid monohydrate
   (FW = 210.14 g/mol)

2. Determine the volume of buffer required for the antigen retrieval. Prepare 10mM sodium citrate by diluting 0.1M sodium citrate with DI. Adjust the buffer to pH 6.0 with the 0.1M citric acid.
3. If needed, transfer the 10mM sodium citrate buffer to a retrieval container and preheat the buffer to 90°-100°C.
4. The heat source needs to be preheated prior to use; refer to the manufacturer's manual.
5. Place the slides into the container with the preheated retrieval buffer and place in the heat source for 15-20 minutes. Adjust the incubation time as needed.
6. Remove the container from the heat source and allow the slides to cool at room temperature for 15-20 minutes.
7. Continue with the immunostaining protocol.