

## **The protocols of antigen retrieval (for formalin-fixed, paraffin-embedded, FFPE tissue sections)**

### **1. High temperature methods. You may use either one of the methods below.**

#### **Method 1: Microwave Oven**

Heat the buffer (0.01M sodium citrate buffer, pH 6.0) to boiling, then put the array slide in the buffer and repeat boiling process in the microwave oven. This method is suitable for the following antigen retrieval: AR, Bax, Bcl-2, C-fos, X-jun, C-kit, C-myc, E-cadherin, Chromogranin A, Cyclin, ER, Heat shock protein, HPV, Ki-67, MDMZ, p53, p34, p16, p15, P-glycoprotein, PKC, PR, PCNA, ras, Rb, and Topoisomerase II, et al. Higher pH, such as pH 12.0 can also be tested if it does not work.

#### **Method 2: Pressure Cooker**

Heat the buffer (0.01M sodium citrate buffer, pH 6.0) to boiling by using a stainless steel pressure cooker. Do not lock lid at this moment. When the buffer boils, put a metal rack with array slides into pressure cooker and make sure array slides are well immersed in buffer for 5 min, then lock the lid and put the small valve in open position, boiling array slide for another 10 min. Remove pressure cooker from heat source and cool it down under cold running water. When the small valve sinks, open the lid and remove array slides. This method can be used for the antigens such as nuclear antigens that are difficult to be examined later due to formalin-fixation process.

#### **Method 3: Boiling Bath**

Heat the buffer (0.01M sodium citrate buffer, pH 6.0) to about 95°C, and then put array slides in the buffer for 10~15 min. Do not let the water boiling, or it will wash the tissue sections off!

### **2. Enzyme digestion method**

Use either 0.1% trypsin or and 0.4% pepsin. Put the array slide into trypsin solution and heat at 37°C for 30 min. This method is suitable for the following antigen retrievals: Collagen, Complement, Cytokeratin, C-erbB-2, GFAP, LCA and LN, et al.