

## Routine IHC Protocol

### 1.0 Procedure

#### 1.1. Deparaffinization/Rehydration

- a. Bake slides in an oven at 60 °C for 30 mins to 1 hour.
- b. De-paraffinize and hydrate tissue sections by soaking the slides in:
  - I. Xylene 2 X 5 minutes
  - ii. 100% ethanol, 2 X 5 minutes
  - iii. 95% ethanol, 1 X 3 minutes
  - iv. 70% ethanol, 1 X 3 minutes
  - vi. Distilled water, 1 X 3 minutes

#### 1.2. Peroxidase inactivation

- a. Add 2 drops of the peroxidase blocking solution into each ml of dH<sub>2</sub>O.
- b. Cover the tissue sections with the above solution and incubate the slides at R/T for 10 min.

#### 1.3. Antigen retrieval with heat

Dilute the 10X retrieval with dH<sub>2</sub>O.

#### ***Water bath or rice cooker method (recommended):***

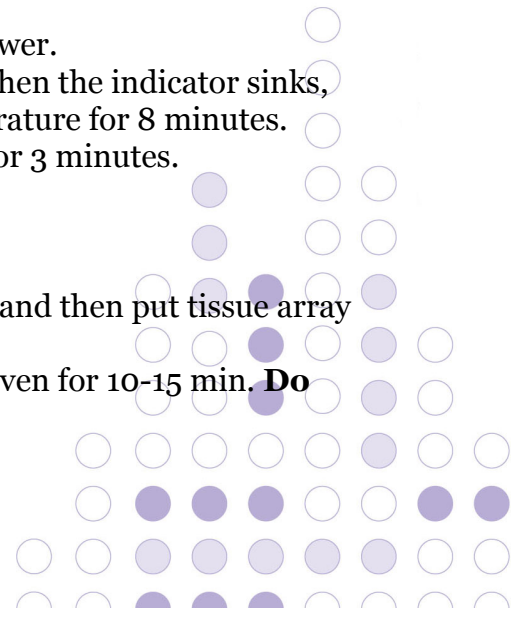
- a. Pre-heat the bath or cooker with heat-resistant jar or box filled with retrieval Buffer to >95°C (do not let the buffer boil actively).
- b. Immerse the slides in the buffer and cover the jar with the lid or plastic clean film.
- c. Continue to heat or "cook" the slides at >95°C for 5-8 minutes.
- d. When water bath or rice cooker is turned off, unplug the cooker and remove the jar to the bench top.
- e. Allow it to cool down for 20 min.

#### ***Pressure cooker method (need more retrieval buffer):***

- a. Heat the antigen retrieval buffer to boiling by using a stainless steel pressure cooker. Do not lock lid at this moment.
- b. When the buffer boils, power off, put a heat-resistant rack with tissue array slides in the cooker and make sure that the buffer covers the slides.
- c. Lock the lid, cook the array slides for 5 minutes with moderate power.
- d. Remove the pressure cooker and cool it down under tap water. When the indicator sinks, open the lid and remove the jar or box and let it cool at room temperature for 8 minutes. Transfer the slides from the jar or box to tap water and rinse them for 3 minutes.

#### ***Microwave oven method:***

- a. Heat the antigen retrieval buffer in a heat-resistant jar to boiling, and then put tissue array slides in the buffer. Cover the jar with lid or plastic clean film.
- b. Set the power to simmer or 1/3 of energy level in the microwave oven for 10-15 min. **Do NOT** let the buffer boil.
- c. Remove the jar and leave it at R/T to cool for 15 minutes.



## 1.4. IHC Detection

- a. Rinse the slides with 2 changes of TBST, 2 minutes each.
- b. Block the slides with the blocking solution for 10 minutes, and then tip off the solution without rinsing or washing.
- c. Cover the tissue array sections with primary antibody diluted in the dilution solution or TBST (100-200µl per section)
- d. Incubate the array sections with the antibody at R/T for 30 to 1 hour in a humidified chamber.
- e. Wash slides three times with TBST (3 min each).
- f. Incubate the sections with Post-blocking for 20 min.
- g. Wash slides twice with TBST (3 min each).
- h. Incubate the sections with the poly-HRP-anti-Rabbit/mouse Ig at R/T for 30 to 50 min. (100-200µl per section).
- I. Wash the slides three times with TBST (3 min. each).
- j. Incubate the sections with the fresh-made DAB substrate solution at R/T until suitable staining develops (generally 2-5 min).
- k. Rinse the slides with running tap water.
- l. Counterstain the sections with Mayer's Hematoxylin (30 to 60 seconds).
- m. Rinse the slides well with running tap water.
- n. Dehydrate the sections through 95% ethanol for 2x2 minutes, 100% ethanol for 2x3min. Clear them in xylene for 2x3min.
- o. Mount slide.

