

## EASY FISH PRETREATMENT KIT

**This pretreatment kit is developed for conventional paraffin-embedded tissues.**

### **Pretreatment:**

Mount 4 - 6  $\mu\text{m}$  formalin-fixed paraffin-embedded tissue sections on positively charged slides.

0. Pre-warm 50 ml Citrate Buffer pH 8 solution at 96-98°C and 50ml of OACP Pepsine Solution at 37°C .

1. Bake mounted slides for 20 mins at 65 °C.

2. De-paraffinize warm slides by soaking in xylene or xylene substitute for two times 10 minutes (min).

3. Re-hydrate by soaking in 100%, 85% and 70% ethanol for 3 min each.

4. Wash with dH<sub>2</sub>O for 3 min at room temperature (RT).

5. Place slides in Citrate Buffer pH 8 at 96-98°C° for 5-30 min (Time depending on tissue fixation and tissue type).

6. Place slides in OACP Pepsine Solution at 37°C for 5-30 min (Time depending on tissue fixation and tissue type), and then wash in dH<sub>2</sub>O for 3 min at RT.

7. Rinse in 2 x SSC (obtained by eluting 10ml of Concentrated Wash Buffer in 990ml of dH<sub>2</sub>O) for 3 min at RT.

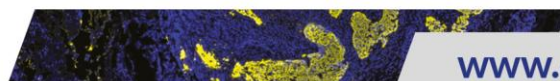
8 Dehydrate slides by soaking 70%, 85%, and 100% ethanol for 1 min each time. Air-dry.

**Note:** Check protein digestion and pretreatment by applying 15  $\mu\text{l}$  DAPI counterstain and evaluate slides using a fluorescence microscope equipped with a DAPI filter. Remove cover slip and soak tissue in 2 x SSC for 2 min and prolong protein digestion if sample is not sufficiently digested. Use a fresh sample and reduce protein digestion time if the sample is over-digested.

### **Post-Hybridization:**

1. Pre-warm 50ml of OACP Stringent Wash to 72-75 °C

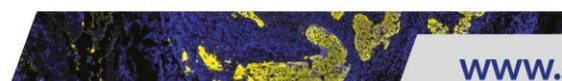
2. Remove rubber cement.





3. Rinse in 2 x SSC (obtained by eluting 10ml of Concentrated Wash Buffer in 990ml of dH<sub>2</sub>O) for 2 min at RT.
4. Place up to 12 slides in the pre-warmed W OACP Stringent Wash for 2 min at 72 °C (+- 3 °C)
5. Rinse in 2 x SSC (obtained by eluting 10ml of Concentrated Wash Buffer in 990ml of dH<sub>2</sub>O) for 2 min at RT.
6. Dehydrate in fresh 70%, 85% and 100% ethanol, incubate for 1 min each at RT. Air dry at RT and proceed to [counterstaining](#)

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