EASY FISH PRETREATMENT KIT

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

Pretreatment:

Mount 4 - 6 µm 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 dH2O 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 dH2O for 3 min at RT.
7. Rinse in 2 x SSC (obtained by eluting 10ml of Concentrated Wash Buffer in 990ml of dH2O) 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 µl 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.

OaCP IE LTD Phoenix House, Monahan Road, T12H1XY Cork (IE)
+353 (0) 212376197 - ask@oacp.it - **VAT:** IE3518703DH
OaCP S.R.L. Viale Fanin, 48 - 40127 Bologna (IT)
(+39) 051 6370834 - ask@oacp.it - **VAT:** IT 03616931204

3. Rinse in 2 x SSC (obtained by eluting 10ml of Concentrated Wash Buffer in 990ml of dH2O) 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 dH2O) 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