FISH protocol for Stripping and Reprobing of FISH slides hybridized with any ZytoLight or FlexISH FISH probe

Test Material

We recommend the following tissue preparation:

- Fixation in 10% neutrally buffered formalin for 24 h at RT.
  In order to achieve optimum and uniform fixation and paraffin embedding, the sample size should not exceed 0.5cm³.
- Standard processing and paraffin embedding
  Use premium quality paraffin. Infiltration and embedding should be carried out at temperatures lower than 65°C.
- Prepare 3-5 µm microtome sections.
  Draw up the sections onto silane-coated or adhesion slides (e.g. HistoBond) and fix at 50-60 °C for 2-16h.
- Perform FISH with any ZytoLight FISH probe respectively FlexISH probe according to the manual of the according FISH Tissue Implementation Kit (Z-2028-5/-20 or Z-2182-5/-20).

Materials and Reagents needed from ZytoVision:

- FISH Probe from ZytoVision (ZytoLight or FlexISH)
- Wash Buffer SSC [WB-0001-500]
- Pepsin Solution [ES-0001-4/-8/-50/-1000] optional
- 25x Wash Buffer A [WB-0002-50]
- 20x SSC Solution [WB-0003-50]
- DAPI/DuraTect-Solution [MT-0007-0.8]
- Formaldehyde Dilution Buffer Set (including 10x $MgCl_2$, 50 ml; 10x PBS, 50ml) [PT-0006-100] optional
- Fixogum

Additional Materials:

- Water bath (37°C, 70°C)
- Neutrally buffered formaldehyde (10% or 37%)
- Hot plate (75°C)
- Hybridization oven (heating oven; 37°C)
- Staining jars, 50-80ml
- Humidity chamber
- Pipet (10µl, 30µl)
- Adhesive pistol, including hot adhesive or rubber cement (Fixogum)
- Ethanol 100%, denatured
- Deionized or distilled water
- Drying block
- Coverslips (22mm x 22mm, 24mm x 60mm)
- Fluorescence microscope

1.1 Preparatory Steps:

Day 1:

• *Preparation of one ethanol series (70%, 90%, and 100% ethanol solutions):*
Dilute 7,9 and 10 parts of 100% ethanol with 3,1 and 0 parts of deionized or distilled water, respectively. These solutions can be stored in suitable containers and can be reused (Day 2).

• *Preparation of 0.1x SSC Solution:* Dilute 1 part 20x SSC Solution **[WB3]** with 199 parts deionized or distilled water. Adjust the pH to 7-7.5 before filling up to the final volume. Fill one staining jar with 0.1x SSC Solution and pre-warm it to 70°C.

• *Wash Buffer SSC **[WB1]:*** Bring to room temperature.

• *Preparation of 1% Formaldehyde solution:* optional For 100 ml 1% Formaldehyde solution mix either 2.7 ml of 37% neutrally buffered formaldehyde or 10 ml of 10% neutrally buffered formaldehyde with 10 ml of 10x $MgCl_2$ **[PT4]** and 10 ml of 10x PBS **[PT5]** and adjust volume to 100 ml with deionized or distilled water. Mix thoroughly.

• FISH Probe to RT.

Day 2:

• *Preparation of 1x Wash Buffer A:* Dilute 1 part 25x Wash Buffer A **[WB2]** with 24 parts deionized or distilled water. Fill three staining jars with 1x Wash Buffer A and pre-warm it to 37°C.

• *Preparation of one ethanol series (70%, 90%, and 100% ethanol solutions).*

• DAPI/DuraTect™-Solution **[MT7]**: Bring to room temperature before use, protect from light.

1.2 Stripping [Day 1]:

- Remove the coverslip by submerging in Wash Buffer SSC **[WB1]** for 5 min.
- Incubate for 10 min in pre-warmed 0.1x SSC Solution at 70°C.

1.3 Proteolysis [Day 1]: optional

In case the FISH slide used for reprobing was underdigested, an additional proteolysis step can be performed at this point:

- Transfer slides to deionized or distilled water, wash for 2x 2 min and drain off or blot off the water.
- Apply (dropwise) Pepsin Solution to the tissue/cell section and incubate for approx. 15 min at 37°C in a humidity chamber.
  Depending on multiple factors, e.g. nature and duration of fixing, thickness of sections, and nature of tissue/cells, different incubation times may be required. As an incubation guideline, we recommend an incubation time of 5-15 min for tissue samples and 0-3 min for cell samples. As a general rule, we recommend to ascertain the optimum time for proteolysis in pre-tests.
- Wash for 5 min in Wash Buffer SSC.

1.4 Post-Fixation [Day 1]: optional

- Incubate for 15 min in 1% Formaldehyde solution and wash for 5 min in Wash Buffer SSC.

1.5 Dehydration [Day 1]:

- Incubate in 70%, 90%, and 100% ethanol, each for 1 min.
- Air dry sections.

1.6 Denaturation and Hybridization [Day 1]:

- Pipette 10 µl FISH probe each onto individual samples.
  A gentle warming of the probe, as well as using a pipette tip which has been cut off to increase the size of the opening, can make the pipetting process easier. Avoid long exposure of the probe to light.
- Avoiding trapped bubbles, cover the samples with a coverslip (22 mm x 22 mm). Seal the coverslip, e.g. with a layer of hot glue from an adhesive pistol or with rubber cement.
- Denature the slides according to the used FISH protocol, e.g. on a hot plate.
  Depending upon the age of the sample and variations in the fixation stage, it may be necessary to optimize the denaturing temperature .
- Transfer the slide to a humidity chamber and hybridize overnight at 37°C (e.g. in a hybridization oven).
  It is essential that the tissue/cell samples do not dry out during the hybridization step.
- **Please note:** F*lex*ISH probes have to be hybridized overnight as well. A 2-hour hybridization is not possible after stripping.

1.7 Post-Hybridization and Detection [Day 2]:

- Proceed with Day 2 of either the Zyto*Light* FISH Tissue Implementation Kit protocol or the F*lex*ISH Tissue Implementation Kit protocol. The used Implementation Kit depends on the used probe.