# FISH Protocol for FHACT™ Probe

Empowering Personalized Cancer Treatment

## Reagent Provided

*For Research Use Only. Not for use in diagnostic procedures.*

Probe: 110 µl per vial. Ready to use.

The probe is premixed in hybridization buffer (formamide, dextran sulphate, SDS and SSC).

**Warning and Precaution:** Handle all reagents and slides containing fluorophores in reduced light to prevent photobleaching. The probe mixture contains formamide, avoid exposure. Handle all reagents with care; wear appropriate personal protective equipment.

## Reagents & Material Required but Not Provided

| | |
|---|---|
| Coplin jar | 1N Hydrochloric acid (HCl) |
| Waterbath | Pepsin |
| Microcentrifuge | 10X Phosphate Buffer Saline (PBS) |
| Centrifuge tube (15 ml) | 20X Saline Sodium Citrate (SSC) |
| Slide tray | 10% Formalin |
| Rubber cement | 100% Ethanol |
| Cover glass | Tween 20 |
| Forceps | DAPI/Antifade |
| Temp. controlled hot plate | Autoclaved distilled water |
| Micropipettes (1-200 µl) | Acetic acid |
| $MgCl_2$ | Methanol |

## Reagent Preparation

**2X SSC.** Mix 100 ml of 20X SSC and 900 ml of distilled water. Store at room temperature (RT).

**2X SSC/0.1% Tween 20.** Add 100 ml of 20X SSC and 1 ml of Tween 20 to 900 ml distilled water. Mix well by swirling. Store at RT.

**0.5X SSC/0.1% Tween 20.** Add 25 ml of 20X SSC and 1 ml of Tween 20 to 975 ml distilled water. Mix well by swirling. Store at RT.

**Ethanol Series (70%, 85%, and 100%).** Prepare v/v dilutions of 100% ethanol with distilled water. Store at RT.

**0.01N HCl.** Add 0.5 ml of 1N HCl to 49.5 ml distilled water.

**0.4% Pepsin Stock Solution (4 mg/ml).** Dissolve 250 mg of pepsin in 62.5 ml of 0.01 N HCl. Store 2 ml aliquots at -20°C.

**1X PBS.** Mix 100 ml of 10X PBS to 900 ml distilled water. Adjust pH to 7.0. Store at RT.

**Formalin Fixative.** Add 12.5 ml of 10% formalin to 37 ml of 1X PBS and 0.5 ml of 100X $MgCl_2$. Store at RT.

**3:1 Fixative.** Add 15 ml of methanol to 5 ml acetic acid. Make fresh each time, discard unused portion.

## Filter Requirements

| Fluorophore | $Excitation_{max}$ | $Emission_{max}$ |
|---|---|---|
| DAPI | 360 nm | 460 nm |
| Aqua | 431 nm | 480 nm |
| Green | 496 nm | 551 nm |
| Gold | 525 nm | 551 nm |
| Red | 580 nm | 603 nm |

**Storage conditions:** Hybridized slides and Probe must be stored at -20°C protected from direct light.

## Protocol

**Ready to use product.**

**Do not reconstitute or dilute with hybridization buffer.**

### Slide Preparation

1. Transfer the cervical cells stored in liquid-based medium to a 15 ml centrifuge tube and centrifuge at 1,200 rpm for 10 min at RT.
2. Discard the supernatant and resuspend the pellet in freshly made 3:1 fixative.
3. Repeat the wash process (centrifuge-resuspend in fixative), about 2-3 times to clear the cell pellet of all debris. Resuspend in 2 ml of fixative.

*Procedure Note: Some specimens may require more or less fixative depending on the pellet size.*

4. Prepare the slide according to the standard laboratory protocol. If not hybridizing within a few days, store the slide at 4°C, or -20°C for long-term storage (over a month).

### Slide Pretreatment with Pepsin

1. Dilute the pepsin stock to a final concentration of 0.5 mg/ml using 0.01 N HCl. Incubate the slide for at least 10 min at 37°C.

*Procedure Note: Some specimens may require longer digestion time in pepsin.*

2. Wash the slide 5 min in 1X PBS at RT.
3. Incubate the slide for 5 min in 1% formaldehyde fixative at RT.
4. Wash the slide for 5 min in 1X PBS at RT.
5. Dehydrate the slide in 70%, 85%, and 100% ethanol at RT for 1 min each. Air dry.

*Procedure Note: Check the morphology of the sample under phase contrast microscope before hybridization.*

### Probe Denaturation / Hybridization

1. Vortex the Probe briefly and spin the tube in a microcentrifuge.
2. Apply 10 µl of the Probe, cover with a cover glass (22 x 22 mm).

*Procedure Note: Care should be taken to avoid air bubbles. Smaller or larger cover glass may be used with proportional change in Probe volume.*

3. Seal the edges of the cover glass thoroughly with rubber cement.
4. Co-denature the slide and the Probe mixture for 3 min at 80°C on a temperature controlled hot plate protected from direct light.
5. Incubate for 48 hours in a humidified environment at 37°C and protected from light.

### Post Hybridization Washing

*Procedure Note: Do not allow the slide to dry before the washes are complete.*

1. Remove the rubber cement with forceps.
2. Remove the cover glass by soaking the slide in 2X SSC at RT.
3. Wash the slide 2 x 5 min in 2X SSC/0.1% Tween 20 at 45°C with agitation.
4. Briefly rinse the slide in distilled water and air dry the slide out of direct light.
5. Apply 20 µl of DAPI/Antifade solution to the hybridized area and cover with a cover glass (25 x 25mm).

*Note: Dilute the DAPI to 1:10 with Antifade to prevent the counterstain from overwhelming the intensity of the blue signal.*

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