ISH Tissue Pretreatment kit: IS0003

Intended Use: For Research Use Only. This product is intended to be used in heat and enzyme pretreatments of formalin-fixed, paraffin-embedded (FFPE) tissues prior to in situ hybridization (ISH).

Package:

Description	Catalog No.	Size
ISH Tissue Pretreatment kit	IS0003	1000ml / 10ml

Reagents Supplied:

Reagent A: One bottle of ready to use 1000 ml Heat Pretreatment Solution.
Reagent B: One bottle of ready to use 10 ml Enzyme Pretreatment Reagent.

Storage: Store at 2°- 8°C. Do not freeze. All performance claims are void after the kit expiration date.

Materials Required But Not Supplied:

FFPE tissue section Reagent water Heating Instrument.

Procedure: For use after deparaffinizing and rehydrating slides. If necessary, block endogenous peroxidase activity before HIER step.

1. Wash slides in 3 changes of reagent water to remove alcohol/peroxidase block.
2. Heat the Tris-EDTA Buffer (Reagent A) in a beaker on a hotplate until it is steadily boiling, and at ≥ 98°C. To prevent buffer from evaporating, the beaker should be covered with either a glass cover or aluminum foil.
3. Place slides in the boiling solution, cover the beaker, and boil for 15 min.
4. Transfer slides immediately to dH2O at RT (15-30°C) and wash three times, 2 min. each.
5. Wash slides in 3 changes of reagent water at room temperature.
6. Cover tissue with 100-200 µl of Reagent B Enzyme Pretreatment Reagent for 5 – 10 minutes at 37°C or 15 – 20 minutes at room temperature.
   Note: Depending tissue fixative used, different incubation times may be required. Excessive enzyme pretreatment will cause loss of nuclei structure. See Precautions section below.
7. Wash slides in 3 changes of reagent water at room temperature.
8. Dehydrate slides in a series of 70%, 85%, 95%, and 100% ethanol for 2 minutes each at room temperature, air dry at room temperature for at least 20 minutes, and proceed to denaturation and hybridization.

Precautions:

For professional users only.

Heat Pretreatment and Enzyme Pretreatment is the most critical step for successful ISH performance. The FFPE tissue sections must be boiled or heated above 98°C for 15 minutes in Heat Pretreatment Solution. Different enzyme incubation time (3-15 minutes) may be required, depending on tissue type and fixation method. For most tissues using standard fixation procedure, 5-10 minutes enzyme pretreatment at 37°C or 15-20 minutes at room temperature will produce the best ISH results. Be sure to pre-warm the Enzyme Pretreatment Reagent to room temperature prior to adding to the tissue section.

Excessive epitope retrieval of FFPE tissues could result in damage of tissue morphology or tissue sections becoming detached from the slide. Inadequate epitope retrieval of FFPE tissue could result in weaker staining. Enzyme pretreatment of the tissue section should be evaluated first before signal enumeration and scoring. If nuclei are not counterstained and there is an absent or very weak ISH signal, this may be due to nuclear loss as the result of excessive enzyme pretreatment. If nuclei counterstain is strong but ISH signal is absent in the nuclei, this may be due to under pretreatment of enzyme.

Throughout the entire procedure, unless otherwise indicated, it is important that the tissue section does not dehydrate.

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