

# FFPE Pre-Treatment Kit I

GeneCopoeia Inc.  
9620 Medical Center Drive, Suite 101  
Rockville, MD20850, USA  
Tel: +1(301)762-0888;  
Toll free: +1(866)360-9531  
Fax: +1(301)762-3888  
Web: www.genecopoeia.com

## I. Kit components

Name	Cat#	Size	Shipping	Store
Pretreatment Solution	FP202-010	5 ml (100x)	Ice pack	4°C. Stable for 6 months. -20°C. Stable for at least 1 year.
Protease I	FP203-010	5 ml (1x)	Ice pack	

**Note 1:** For long term storage, the **Protease I** Solution can be aliquotted and stored at -20°C. 1-2 cycles of freeze-thaw will not adversely affect the protease activity.

**Note 2:** The frozen **Proteinase I** should be thawed at 4°C before use. Some precipitate may appear after thawing, and placing the **Proteinase I** on ice for 15-20min with gentle and intermittently shaking should completely dissolve the precipitate. Do not vortex.

## II. Procedures

### Laboratory Reagents, Materials and Equipment:

- Ethanol (100%), 20xSSC, NP-40, Hemo-De (or Xylene), adjustable pipettes and tips, Timer, Diamond-tipped scribe, Forceps, Coplin slide jars (50 mL), thermometers, Vortex mixer, Positively-charged glass slides, Slide box with lid, Microcentrifuges, Slide warmer, Incubator (37°C), Purified water bath ( 40 ± 2°C). Circulating water bath (85± 1°C), Microtome.

### Specimen Collection and Processing:

**Note:** Exposure of the specimens to acids, such as decalcifying agents, strong bases, and extreme heat should be avoided. Such conditions are known to damage DNA and may result in FISH assay failures. Use tissue specimens that were fixed in formalin (10% neutral buffered formalin) and that are well processed and produce good tissue sections. The preferred fixation duration for tissue samples is 12 to 48 hours

- Cut serial 3-4 µm thick paraffin sections using a microtome.
- Float the sections on the surface of a purified water bath set at 40 ± 2°C.
- Mount the sections in the same direction on positively-charged glass slides.
- Allow the slides to air-dry and then bake the slides on a slide warmer at 56°C for 30 min.
- Store prepared slides at -20°C.

### De-paraffinization

- Bake FFPE slides at 56°C for 4 hrs to overnight
- Use a diamond-tipped scribe to mark the specimen area on the FFPE slides.
- Immerse slides in three changes of Hemo-De (or Xylene), each for 5 min at ambient temperature
- Dehydrate slides in two changes of 100% ethanol, each for 5 min. at ambient temperature

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- Dry slides at 56°C on slide warmer

### Slide Pretreatment

- Prepare 50ml of **1x Pretreatment Solution** in a Coplin jar
- Pre-warm to  $85 \pm 1^\circ\text{C}$
- Incubate slides in the pre-warmed **1x Pretreatment Solution** for 60-90 min at  $85 \pm 1^\circ\text{C}$
- Immerse slides in 2xSSC at room temperature twice for 1min.
- Immerse slides in purified water for 1 min.
- Air dry slides.

### Protease Pretreatment.

- Warm up the slides in a moisture chamber at 37°C for at least 10 min
- Pipette enough volume (400~500µl / slide) of **Protease I** solution to cover the specimen or desired area on each slide.

**Note:** The **Protease I** solution should be kept at 4°C or on ice. It does not need to warm up before use.

- Incubate at 37°C for 10 - 20 min.  
**Note:** Incubation times could be varied for different tissues. The optimal incubation time may vary from 5 to 40min depending on type of tissue, fixation time and/or thickness of tissue, and should be determined by the user.
- Tap off the **Protease I** solution and immerse slides in 2xSSC at room temperature for 2min.
- Dehydrate slides at 70%, 90%, 100%Ethanol, each for 2min,
- Air dry slides,
- Proceed to FISH, or store at -20°C for several days if not using immediately.