

KA2375 Protocol

Serum protocol for FFPE FISH PreTreatment Kit (KA2375)

FFPE FISH PreTreatment Protocol (KA2375): https://www.novusbio.com/products/ffpe-fish-pretreatment-kit_ka2375
Introduction

Pre-treatment is an essential step for effective FISH on Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections. This kit contains key reagents used to pretreat FFPE tissue sections from dewaxing with xylene through proteolytic digestion to help permeabilization of cell membranes to facilitate penetration of fluorescence labeled probes. After proteolytic digestion, proceed the subsequent steps with the FISH assay protocol.

Material and Method

A. List of component

1. 1x Paraffin Pretreatment Solution, 500 mL
2. Protease (Pepsin), 0.5 ml x 10 vials

B. Materials Required But Not Provided

1. 2xSSC (Saline-sodium citrate buffer): 0.3 M NaCl, 0.03 M sodium citrate, pH 7.0
2. Xylene
3. 100% Ethanol
4. 70% Ethanol
5. Protease Buffer (0.2N HCl, pH 1)

C. Preparation of Reagents

1. Protease Solution: Add 500 uL of Protease in 50 mL Protease Buffer and mix gently.

D. Stability and storage

The kit is shipped at 4 degrees C. After arrival, please store the 1x Paraffin Pretreatment Solution at room temperature (RT), and store the Protease (Pepsin) at -20 degrees C.

E. Procedures

1. Deparaffinizing Slides

- a. Immerse slides in Xylene for 5 minutes at room temperature, repeat three times. (Use fresh Xylene each time.)
- b. Dehydrate slides in 100% Ethanol for 5 minutes at room temperature. Repeat once more.
- c. Air dry slides or place slides on a 45 - 50 degrees C slide warmer.

2. Pretreating Slides

- a. Immerse slides in 1x Paraffin Pretreatment Solution at 95 degrees C for 30 minutes.
- b. Immerse slides in 2xSSC (Saline-sodium citrate buffer) for 5 minutes. Repeat once more.

3. Protease Treatment (proteolytic digestion)

- a. Immerse slides in Protease Solution at 37 degrees C for 10 - 20 minutes.
- b. Immerse slides in 2xSSC for 5 minutes. Repeat once more.
- c. Immerse slides in 70% ethanol at room temperature for 1 min.
- d. Remove the slide, and immerse in 100% ethanol at room temperature for 1 min.
- e. Air dry slides or place slides on a 45-50 degrees C slide warmer.

4. Please follow the subsequent steps indicated in FISH protocol, which are in the second page (Paraffin embedded tissue) of FISH assay http://www.abnova.com/protocol_pdf/FISH.pdf target="_blank"> procedures .