This protocol is intended as a guide only, for full experimental details please read the product user manual.

Fresh Frozen Tissue Sample Preparation for Single-plex RNAscope™ 2.5 and BaseScope™ Chromogenic Assays

Introduction

Use this Technical Note to prepare Fresh Frozen Tissue for single-plex RNAscope™ and BaseScope™ Chromogenic assays. For Part 2 of the detection assay procedures, refer to the specific RNAscope™ or BaseScope™ Chromogenic Detection assay manual available on the ACD website. See the Safety Data Sheet (SDS) available in document download section on each product page.

Part 1: Prepare the Tissue Sections

Section Preparation

1. Cryosection the tissue to 10—20µm thickness and place onto SuperFrost Plus slides. Store slides at room temperature.
2. Keep the sections at –20°C to dry for 1 hour.
3. Store the sections at –80°C.
4. Sections may be stored up to 3 months at –80°C.

   NOTE: Do not process the slides with any fixative (alcohol or formaldehyde) before this step. The slides can be shipped on dry ice.

Sample Fixation

1. Pre-chill 200 mL of 10% neutral buffered formalin (NBF) or 4% paraformaldehyde (PFA) in 1X PBS to 4°C.
2. Remove fresh frozen tissue slides from –80°C. Immediately immerse the slides in the pre-chilled 10% NBF or 4% PFA. 3. Incubate the slides for at least 15 MIN at 4°C.

Dehydrate the Tissue

1. Prepare 200 mL 50% EtOH, 200 mL 70% EtOH, and 400 mL 100% EtOH.
2. Remove the slides from NBF or 4% paraformaldehyde. Immerse in 50% EtOH. Incubate for 5 MIN at ROOM TEMPERATURE (RT).
3. Remove the slides from 50% EtOH. Immerse in 70% EtOH. Incubate for 5 MIN at RT.
4. Remove the slides from 70% EtOH. Immerse in 100% EtOH. Incubate for 5 MIN at RT.
5. Remove the slides from 100% EtOH. Immerse in fresh 100% EtOH. Incubate for 5 MIN at RT.
6. Store the slides in 100% EtOH at –20°C for up to 1 WEEK. Prolonged storage may degrade sample RNA.

Dry the Slides

1. Remove slides from 100% EtOH. Leave slides for 5 MIN at RT.
2. Draw 2—4 times around tissue using the Immedge™ hydrophobic barrier pen. Let the barrier dry completely ~1 MIN.

Part 2: Tissue Pretreatment

Apply RNAscope™ Hydrogen Peroxide and Protease IV

1. Add 2—4 drops/slide of RNAscope™ Hydrogen Peroxide for 10 MIN at RT then rinse once with 1XPBS.

2. Take slides from the Tissue-Tek® Slide rack, and add 2—4 drops of RNAscope Protease IV to each section. Incubate for 30 MIN at RT.

3. Wash slides with 1X PBS by moving the rack up and down 3—5 times and repeat with 1X PBS.

   IMPORTANT: Use enough solution to completely cover the sections.

   NOTE: Some tissues may require different treatment time (15–30 MIN) with Protease IV. Always start with 30 MIN and adjust based on signal and morphology.

   IMPORTANT: Proceed to the RNAscope™ or BaseScope™ protocol using the appropriate Part 2 Chromogenic Detection User Manual* available at http://www.acdbio.com/technical-support/user-manuals.

   *RNAscope™ 2.5 HD Detection Reagents-Brown User Manual, Part2 (Doc. No. 322300-USM); RNAscope™ 2.5 HD Detection Reagents Red User Manual, Part 2 (Doc. No. 322350-USM); BaseScope™ Detection Reagent Kit-RED User Manual (Doc. No. 322900-USM).

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