Protocol 1140

For Preparation Of Tissue Sections

1140 Preparation of tissue sections
Version 8.1

Contents

1 INTRODUCTION

1.1 Intended use

This protocol describes a method for cutting tissue sections of biopsies or tissues for application on PamChip® kinase profiling arrays.

2 PROTOCOL

2.1 Equipment

1. Microtome
2. Disposable sterile latex or nitrile surgical gloves
3. Sterile 0,5-2 ml Eppendorf tubes
4. Cryoblock for Eppendorf tubes
5. Injection needle

2.2 Safety & precautions

For materials and handling microtome: see suppliers' information.

2.3 Cleaning tools and equipment

Since protein, not RNA, will be extracted, there is no risk of contamination between samples. Cleaning between samples can be less rigorous.

2.4 Procedure

1. Pre-cool the vials for 1 hour in a Cryoblock at –80°C.
   Note: Pre-cooling the vials will prevent tissue sections from sticking or melting onto the inner surface of the vial.
2. Pre-cool the injection needle for 1 hour in the microtome at –20°C.
3. Place the Cryoblock with vials, precooled at –80°C, in the microtome at –20°C.
4. Ensure biopsy sample and tissue are kept at lowest temperature possible (at least at –20°C or lower).
   Note: Tissue-Tek® OCT™ embedded material is not compatible with PamChip® analysis. If Tissue-Tek® OCT™ Compound[1] is used, cut away as much as possible. Final sections must contain less than 10% Tissue-Tek®.
5. Stick the tissue block with a drop of ultrapure water to the block holder in the microtome
6. Optional: cut one 5 µm tissue section for histological evaluation (HE section pre).

[1] Tissue-Tek® OCT™ Compound: Optimal Cutting Temperature embedding medium, brand name Tissue-Tek, https://www.sakura.eu/Our-products/item/11/Cryotomy/48/Tissue-Tek-OCT-Compound-and-Cryomolds

7. Cut one 60 µm tissue section (optionally: 2 x 30 µm or 6 x 10 µm sections[2]) of ~5 x 5 mm from the snap frozen in liquid nitrogen (or OCT™ embedded) specimen.
   As a guideline, 1.5 mm^3 of tissue should be collected per sample.

Biopsy sample	*Sections*	*Tissue volume*
5 x 5 mm tissue block	1 x 60 µm	1.5 mm^3
3 x 3 mm tissue block	3 x 60 µm	1.6 mm^3
6 mm punch biopsy	1 x 60 µm	1.7 mm^3
3 mm punch biopsy	4 x 60 µm	1.7 mm^3

   Notes:
   - The integrity of the tissue sections is less important than in histology, since protein will be extracted from the sections.
   - Fine needle and scopic biopsies can be lysed without cutting of tissue sections.
8. Place the 60 µm section(s) at the bottom of a pre-cooled vial using the pre-cooled needle. Make sure to keep the section(s) frozen during handling.
9. Optional: cut one 5 µm tissue section for histological evaluation (HE section post).
10. Store the Cryoblock with vials containing tissue sections and leftover tissue at –80°C and record storage details.

2.5 Waste disposal

For materials and handling microtome: see suppliers' information.
Use the accepted internal procedures for disposal of tissue residues.

2.6 Quality control

Histological data (if possible)

1. Perform HE staining on one section, either a section in the middle, or first and last section. Determine the percentage of tumor in the section(s).
2. Mention occurrence of necrosis or other abnormalities in tissue sections.3.
3. Optional: The percentage of Tumor Infiltrating Lymphocytes can be counted in HE stained sections as described in Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015; 26: 259-71.

[2] Thicker tissue sections are easier to handle and result in a more robust sample preparation procedure.

3 TRANSPORT OF SAMPLES

For studies conducted by PamGene, please contact PamGene before preparing samples about exact amounts needed for specific studies.
In case of shipment to PamGene, the samples need to be clearly labelled and packed in a sufficient amount of dry ice accompanied by a list of samples, with preferable an available tracking number.

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4 SUPPORT

For questions contact our support team.

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5 RIGHTS AND RESTRICTIONS

5.1 Disclaimer

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