Protocol

Cryosectioning Tissues

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This protocol was adapted from "Preparation of Cells and Tissues for Fluorescence Microscopy," Chapter 4, in *Basic Methods in Microscopy* (eds. Spector and Goldman). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2006.

INTRODUCTION

Cryosections are rapidly and relatively easily prepared prior to fixation, and they provide a good system for visualizing fine details of the cell. Although cryosections are physically less stable than paraffin- or resin-embedded sections, they are generally superior for the preservation of antigenicity and therefore the detection of antigens by microscopy. The preparation of cryosections does not involve the dehydration steps typical of other sectioning methods, and, furthermore, sectioning, labeling, and observation of specimens can usually be carried out in one day. In general, the sample is frozen quickly in either isopentane or liquid nitrogen. (Small samples such as cells and small tissues may be mixed in a slurry of an inert support medium such as optimal cutting temperature [OCT] compound before freezing). Rapid freezing reduces ice crystal formation and minimizes morphological damage. Frozen sections may be used for a variety of procedures, including immunochemistry, enzymatic detection, and in situ hybridization. A protocol for cryosectioning is presented here.

RELATED INFORMATION

Related procedures for preparing tissues are described in the following *CSH Protocols* articles: **Fixation and Permeabilization of Cells and Tissues** (Fischer et al. 2008a) and **Preparation of Slides and Coverslips for Microscopy** (Fischer et al. 2008b).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

<**!**>Fixative (e.g., formaldehyde; see **Fixation and Permeabilization of Cells and Tissues** [Fischer et al. 2008a])
<**!**>OCT (Tissue-Tek; Sakura Finetek USA) or M-1 medium (Thermo Scientific) (see Step 1)
<**!**>Stain (e.g., toluidine blue [1%-2% w/v in H_2O], hematoxylin, and eosin, or other aqueous stain)
Tissue sample (fresh)

Equipment

Brush (camel hair; optional; see Step 3)
Container for tissue sample storage (see Step 6)

Cryostat with metal grids

Cryostats are essentially a –20°C freezer enclosing a microtome. They are expensive, but in many medical centers, hospital pathology laboratories have cryostats that can be rented.

Slides, microscope

Slides can be poly-L-lysine-coated or silanized (see Step 3 and ***Preparation of Slides and Coverslips for Microscopy*** *[Fischer et al. 2008b])*

Tissue mold, plastic or metal (e.g., Fisher Scientific,VWR)

Tissues, moistened

METHOD

1. Freeze a fresh, unfixed tissue sample, up to 2.0 cm in diameter, in OCT in a suitable tissue mold. Freeze the OCT containing the tissue onto the specialized metal grids that fit onto the cryostat.

   OCT is viscous at room temperature and miscible with H_2O, but freezes into a solid support at –20°C.

   Certain soft tissues, such as brain, are optimally frozen in M-1 medium at –3°C.

2. Cut sections 5-15 µm thick in the cryostat at –20°C. If necessary, adjust the temperature of the cutting chamber ±5°C, according to the tissue under study.

   A camel hair brush is useful to help guide the emerging section over the knife blade.

   See Troubleshooting.

3. Within 1 min of cutting a tissue section, transfer the section to a room temperature microscope slide by touching the slide to the tissue.

   The tissue section will melt onto the slide. This must be accomplished within 1 min of cutting the section to avoid freeze-drying of the tissue.

   Poly-L-lysine-coated or silanized slides improve the adherence of the section.

4. To evaluate tissue preservation and orientation, stain the first slide of each set with toluidine blue (1%-2% w/v in H_2O), hematoxylin, and eosin, or any aqueous stain.

5. Immediately immerse the slide into an appropriate fixative (see **Fixation and Permeabilization of Cells and Tissues** [Fischer et al. 2008a] for guidelines on choosing an appropriate fixative).

   To maximize the adherence of the section to the slide, some researchers allow the section to air-dry onto the slide at room temperature before fixing the sample. The disadvantage of this is that surface tension forces distort the cells, causing loss of high-resolution detail. Air-drying may also cause some changes in immunostaining results.

6. Cover any unused tissue with a layer of OCT to prevent freeze-drying and store the rest of the sample at –70°C.

   For long-term storage, a moistened tissue should be added to the container with the block to prevent desiccation (particularly in a frost-free freezer).

TROUBLESHOOTING

Problem: The tissue is difficult to section.
[Step 2]
Solution: Consider the following:

1. If the tissue frozen in OCT does not cut in a smooth thin sheet, the knife is probably dull.
2. Watery tissues, fatty tissues, or tissues with variable textures are difficult to section.

REFERENCES

Fischer, A.H., Jacobson, K.A., Rose, J., and Zeller, R. 2008a. Fixation and permeabilization of cells and tissues. *CSH Protocols* doi: 10.1101/pdb.top36.

Fischer, A.H., Jacobson, K.A., Rose, J., and Zeller, R. 2008b. Preparation of slides and coverslips for microscopy. *CSH Protocols* doi: 10.1101/pdb.prot4988.

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Cold Spring Harb Protoc; doi: 10.1101/pdb.prot4991