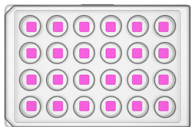
CRYOSECTIONING OF RASTRUM™ REMOVABLE MODELS

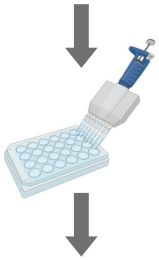
Graphical protocol

1. Take out the cultured RASTRUM Removable Models from the incubator

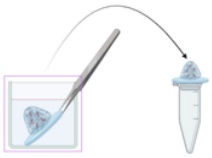


Removable model printed in 24WP containing glass coverslips

2. Wash with phosphate buffered saline (PBS)



3. Transfer coverslips to the lid of a microcentrifuge tube



4. Slide a floss-pick (or scalpel / cell scraper) along the surface of the coverslip and underneath the model to detach it from the glass surface

5. Embed 3D models in OCT and snap-freeze

6. Section and place on microscope slide

7. Stain and image

Introduction

Cryosections offer a rapid and relatively straightforward sample preparation, making them ideal for visualising intricate cellular details. While cryosections may be physically less stable compared to sections embedded in paraffin or resin, they generally excel in preserving antigenicity, crucial for antigen detection through microscopy. Cryosection preparation doesn't involve the dehydration steps found in other sectioning methods, and sectioning, labelling, and specimen observation can be completed in a single day. Frozen sections are versatile and can be used for various procedures, including immunochemistry, enzymatic detection, and in situ hybridisation. Here we provide a protocol to cryosection RASTRUM 3D cell models to easily adopt this technique.

Protocol

For histology applications, it is recommended to use our Removable Model where 3D cell models are printed on top of a coverslip inside a 24-well plate and can be easily removed from the well using tweezers.

Removal and preparation of models

1. Remove and discard the cell culture medium from RASTRUM Removable Models printed onto coverslips in a 24 well-plate.

   Note: *For all fluid removal steps, tilting the plate at a 45-degree angle to collect liquid at the bottom edge of the well will help maximise fluid removal from the plate. When aspirating media, be careful not to pierce or scratch the cell models and inert base with your pipette tip.*

2. Wash the 3D cell models by adding 400 µL of PBS to each well and incubating the plate on an orbital shaker at 900 rpm for 5 minutes at room temperature (RT).

3. Remove and discard the PBS.

Cryo-embedding

1. Using a needle, lift the edge of the coverslip and remove coverslip by tweezers. Transfer to the closed lid of a 1.5 mL microcentrifuge tube.
2. Holding the coverslip in place with tweezers, use a floss pick to slide under the 3D cell model to lift it off from coverslip and transfer to a petri dish containing PBS.

Note:

Please refer to Handling of RASTRUM Removable Model protocol for detailed instructions and tips on handling of these models.

3. Pour Optimal Cutting Temperature compound (OCT) slowly inside the tissue mold without creating any bubbles.
4. Using a spatula with flat head, gently place your models inside the OCT.

   Note: *You may need to orient your sample at this stage.*

5. Snap-freeze the samples in liquid nitrogen or a mixture of dry ice and ethanol. Your samples are now ready for cryo sectioning and staining as usual.

Sectioning

1. Cut sections of 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 type.
2. Within 1 minute 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.

   Note: *Poly-L-lysine-coated or silanised slides improve section adherence.*

3. Cover any unused tissue with a layer of OCT to prevent freeze-drying and store the remaining sample at -70°C.
4. Proceed with your staining as usual.

Reagents and consumables required

Product name	Catalogue number	Company
RASTRUM™ Removable Model	*inquire for details*	*Inventia Life Science*
0.5 mL G29 insulin syringe	*324901*	*BD*
Horn spatula	*Multiple options*	
Tweezers	*Multiple options*	
Dental floss picks (or cell scraper/scalpel)	*Multiple options*	
Low-binding pipette tips (Optional)	*Multiple options*	
4 % paraformaldehyde	*Multiple options*	
1 x phosphate-buffered saline (PBS)	*Multiple options*	
Optimal Cutting Temperature (OCT) compound	*Multiple options*	
Tissue mould (plastic)	*Multiple options*	
Microscope Slides (poly-L-lysine-coated or silanised slides)	*Multiple options*	

Equipment required

Product name	Company
Cryostat with metal grids	*Multiple options*

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