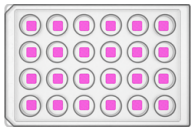
PROCESSING AND PARAFFIN-EMBEDDING OF RASTRUM™ REMOVABLE MODEL

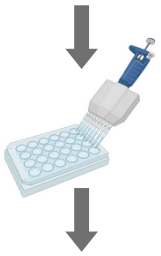
Graphical protocol

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

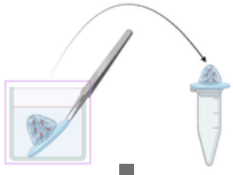


Removable Model printed in 24WP containing glass coverslips

2 Wash, fix and permeabilise for 2 h at RT



3 Transfer coverslips to the lid of a microcentrifuge tube

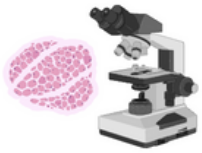


4 Slide a floss-pick (or scalpel / cell scraper) along the surface of the coverslip and underneath the model to detach it from the glass surface

5 Embed 3D models in agarose and process

6 Paraffin embed, section and stain

7 Image



Introduction

This protocol outlines a method to perform immunohistochemistry on formalin-fixed paraffin embedded RASTRUM™ Removable Models. This protocol takes two days and is designed for RASTRUM Removable Model printed onto coverslips in a 24 well-plate.

Protocol

Reagent preparation

1. Prepare fresh solution of 4% paraformaldehyde.
2. Prepare a 1% low gelling temperature agarose solution following manufacturer instruction.
3. Prepare ethanol solutions of various concentrations of 80 %, 90 %, 95 % and 100 % in distilled water.

Removable Model fixation

1. Remove and discard the cell culture medium from RASTRUM Removable Model 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 stab 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.
4. Add 400 µL of fixation buffer (4% paraformaldehyde) and incubate for 1 hour at RT.
5. Remove and discard the fixation buffer into the appropriate waste stream. Add 400 µL of 70% ethanol and incubate overnight (or at least 1 hour) at 4 °C.

Agarose-embedding:

1. Retrieve plate from 4 °C storage and keep at RT for 20 minutes.

   Note: This will prevent uneven gelling of agarose upon contact with the sample.

2. Meanwhile, prepare a 1% low gelling temperature agarose solution (~500 µL agarose solution per model) by dissolving powder with milli-Q water in a small glass beaker. Microwave at 30 seconds intervals until fully dissolved.
3. Using a needle, lift the edge of the coverslip and remove coverslip using tweezers. Transfer to the closed lid of a 1.5 mL microcentrifuge tube.
4. Holding the coverslip in place with tweezers, use a floss pick to slide under the 3D cell model to lift it off from coverslip.

Note:

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

5. On the upturned lid of the 24-well plate, pipette ~150 µL of the molten agarose to form a disk.

   Note: If the agarose has solidified, microwave for 30 seconds until molten and proceed with embedding.

6. Place the 3D cell model on the centre of the molten agarose. Cover 3D cell models with additional molten (~250 µL) agarose until it is completely encased in a droplet . Allow to set at RT for at least 20 minutes or rest lid over ice (**Figure 1**).

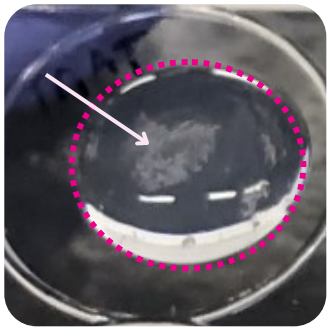


Figure 1. Removable Model embedded in agarose gels. The arrow indicates the Removable Model and the dotted circle shows the agarose gel circumference.

Tissue processing

1. Using a spatula, transfer the agarose gel containing 3D cell model to a pre-labelled plastic histology cassette (**Figure 2**).

Figure 2. Transfer of agarose-embedded 3D model into a plastic histology cassette to proceed with tissue processing.

2. Using 250 mL beaker(s), manually pass the cell models through the following:

   - 80 % ethanol – 45 minutes
   - 90 % ethanol – 45 minutes
   - 100 % ethanol – 45 minutes
   - 100 % ethanol – 45 minutes
   - Xylene – 45 minutes
   - Xylene – 45 minutes
   - Molten paraffin wax – 45 minutes

   Note: If done manually, keep the paraffin molten on wax embedding station.

3. Embed the wax-perfused agarose gel in paraffin to create a block for microtome sectioning.

   Note: You can consider cutting the agarose embedded samples in half to get two semi circles and embed both from the flat surface facing down to have better embedding experience.

4. Section paraffin blocks to generate 5 µm slices on a microtome and transfer them on a 40 °C water bath.
5. Transfer sections to an appropriate microscope slide and allow to dry overnight.

Haematoxylin and eosin staining

1. Incubate microscope slides containing dried sections of paraffin-embedding 3D cell models through the following reagents:

    a. Xylene – 2 minutes
    b. Xylene – 2 minutes
    c. Xylene – 2 minutes
    d. 100 % ethanol – 2 minutes
    e. 100 % ethanol – 2 minutes
    f. 100 % ethanol – 2 minutes
    g. 95 % ethanol – 2 minutes
    h. 70 % ethanol – 2 minutes
    i. Water – 2 minutes
    j. Harris Haematoxylin – 5 minutes
    k. Water – 2 minutes
    l. Acid alcohol – 15 seconds
    m. Water – 2 minutes
    n. Scott's bluing – 2 minutes
    o. Water – 2 minutes
    p. 1 % alcoholic Eosin – 2 minutes
    q. 100 % ethanol – 2 minutes
    r. 100 % ethanol – 2 minutes
    s. 100 % ethanol – 2 minutes
    t. Xylene – 2 minutes
    u. Xylene – 2 minutes
    v. Xylene – 2 minutes

2. Remove slides from xylene and apply coverslip with mounting medium (e.g. DPX).
3. Allow the slide to dry overnight before imaging with a light microscope.

Results

Trophoblasts organoids grown in RASTRUM Removable Model, sectioned at 5 µm thickness showed a successful staining of haematoxylin and eosin (**Figure 3**).

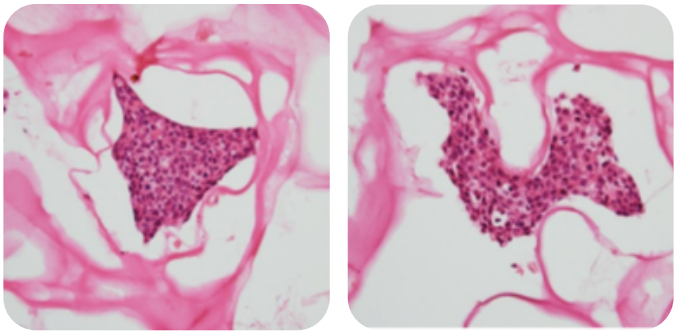


Figure 3. Haematoxylin and eosin staining of RASTRUM 3D cell models. The images were acquired using using Olympus BX51 at 20 X objective.

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*
Tweezers	*Multiple options*	
Horn spatula	*Multiple options*	
Dental floss picks (or cell scraper/scalpel)	*Multiple options*	
Low-binding pipette tips (Optional)	*Multiple options*	
Plastic histology cassettes	*Multiple options*	
Low gelling temperature agarose	*A4018*	*Sigma Aldrich*
4 % paraformaldehyde	*Multiple options*	
1 x phosphate-buffered saline (PBS)	*Multiple options*	
Milli-Q water	*Multiple options*	
80 %, 90 %, 95 % and 100 % ethanol	*Multiple options*	
Xylene	*Multiple options*	
Paraffin wax	*Multiple options*	
Haematoxylin (e.g. Harris)	*Multiple options*	
Acid Alcohol	*Multiple options*	
Scott's Tap Water (Bluing solution)	*Multiple options*	
Eosin	*Multiple options*	
Slide mounting medium (e.g. DPX mounting medium)	*Multiple options*	

Equipment required

Product name	Company
Wax embedding station	*Multiple options*
Microtome	*Multiple options*

info@inventia.life | www.inventia.life

Inventia Life Science Operations Pty. Ltd.

20-22 William Street,
Alexandria, NSW, 2015
Australia