PeptiGel® Protocol:

How to Process PeptiGel® Cultures for Cell Staining

Before you begin, please note:

This protocol describes how to process PeptiGel ® cultures for cell staining including histology, immunohistochemistry and immunofluorescence.

Two options, wax embedding and freezing for cryo-sectioning, are outlined here.

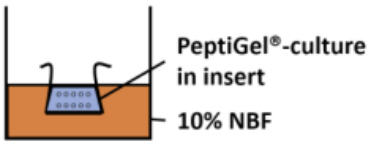
It's recommended to use Greiner Bio-One ThinCerts™ for this protocol. These inserts are made with polystyrene and will dissolve during the xylene wash step.

This protocol has been written as a guide. Therefore, further assay optimization may need to be carried out for your cultures.

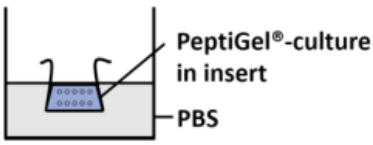
Protocol 1: Wax Embedding

Fixation:

1. Prewarm 10% neutral buffered formalin (NBF) solution to room temperature. Immerse the PeptiGel® (+/- cells) into the solution and incubate for 30 minutes at room temperature (~25°C).

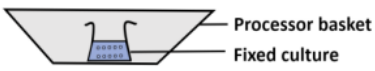


2. Replace the NBF solution with phosphate buffered saline (PBS) solution, enough to immerse the PeptiGel®. Store at 4°C until processing in wax. The maximum recommended storage time is 2 weeks.



Processing in Wax:

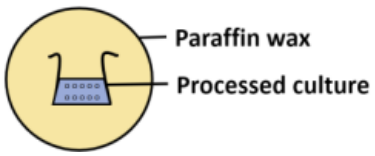
3. Place the fixed insert containing the PeptiGel® cultures (+/- cells) in a cassette. Then place the cassette in a processor basket and assemble into the processor.



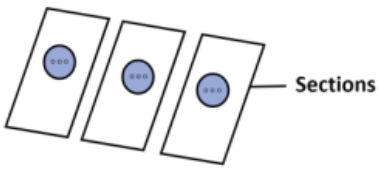
4. Run the wax processing according to the guideline in the table below.

Reagent	Time (seconds)
50% IMS	90
70% IMS	60
99%IMS	60
99%IMS	60
99%IMS	60
99%IMS	60
99%IMS	60
Xylene	90
Xylene	90
Xylene	90
Molten-wax	90
Molten-wax	120

5. Embed the sample according to your instrument's instructions.



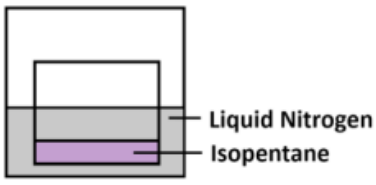
6. Cryo-section according to your instrument's instructions. Individual sections of 5-7 µm thickness are recommended.



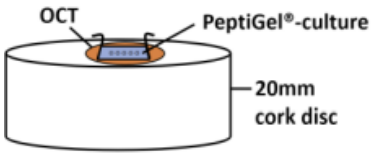
Protocol 2: Freeze for Cryo-Sectioning

Fast Freeze Using Isopentane:

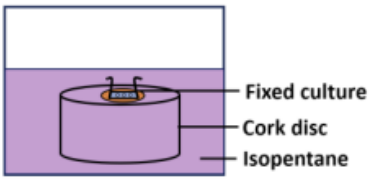
1. Decant liquid nitrogen (LN) into a wide mouthed vacuum flask. Place 25-50 mL of isopentane in a small thick-walled glass beaker and immerse in LN to cool. Lift out when isopentane starts to freeze around the rim of the beaker.



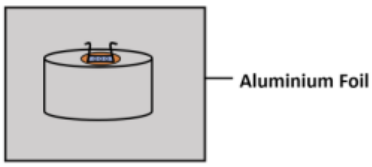
2. Apply a drop of optimal cutting temperature (OCT) compound on to a 20 mm cork disk. Place a PeptiGel® (+/- cells) sample onto the disk and apply more OCT until the sample is covered.



3. Place PeptiGel® (+/- cells) with the disc into the isopentane for 5-15 seconds to freeze, then remove.

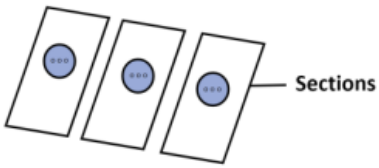


4. Wrap the frozen PeptiGel® in aluminium foil and store at -80°C.



Sectioning Using Cryostat:

5. Cryosection according to the instrument's instructions. Cutting sections of 7-10 µm or cutting a 20 µm thickness if culture samples lack structure, is recommended.



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