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Agarose–gelatin double embedding prior to paraffin processing

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details an agarose–gelatin double embedding workflow to immobilize small tissue fragments or spheroids for routine paraffin embedding. Samples are embedded in a 2% agarose–5% gelatin mixture, then fixed in neutral buffered formalin (NBF) and processed by standard FFPE methods. Compared with conventional methods, it facilitates handling of small specimens, preserves orientation, and minimizes sample damage.

Attachments

PDF

Agarose–gelatin doub...

179KB

Guidelines

Troubleshooting
-Ensure that the agarose–gelatin solution is well mixed and not solidified before embedding.
-Prevent the sample from drying out.
-Process samples in small batches to avoid solidification of the agarose–gelatin gel.
-If the agarose–gelatin gel does not fully cover the sample, add additional solution to embed completely.

Materials

Reagents

- Agarose **Invitrogen** **Catalog #**75510-019
- Gelatin, from porcine skin **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G1890
- DPBS **SolBio** **Catalog #**DPB 001
- Formalin solution, neutral buffered, 10% **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**HT501128

Materials

- 200 µL pipet tip **Corning** **Catalog #**T-200-Y
- 1000 µL pipet tip **Thermo Fisher Scientific** **Catalog #**112NXL-Q
- 50mL Conical Tube **SPL life sciences** **Catalog #**50050
- Petri Dish **SPL life sciences** **Catalog #**10090
- 10mL Serological Pipet **SPL life sciences** **Catalog #**91010
- Microtubes **Corning** **Catalog #**MCT-175-C
- Single Edge Blade **Dorco** **Catalog #**DN-52
- Kimtech Science Wipers **Yuhan-Kimberly** **Catalog #**41112

Equipment

Equipment	
AX1502KR	NAME
ADVENTURER®	BRAND
-	SKU

Equipment

MWO-18M1

NAME

SK Magic

BRAND

-

SKU

Equipment

BW-10E

NAME

Jeiotech

BRAND

-

SKU

Safety warnings

-Exercise caution to avoid burns when handling heated gels.
-During fixation, use appropriate equipment to prevent exposure to fixatives.

Before start

-Ensure all reagents and materials are ready before you begin.
-Verify that the samples are properly prepared.
-Confirm the water bath and microwave oven are working properly.

Procedure

1 Set the water bath to 60 °C .

2 Prepare a [M] 4 Mass / % volume agarose solution(200 µL per sample) : add agarose powder to distilled water (DW) in a 50 mL conical tube, heat in a microwave oven until fully dissolved.

3 Keep the [M] 4 Mass / % volume agarose solution in the 60 °C water bath.

4 Prepare a [M] 10 Mass / % volume gelatin solution: add gelatin powder to a volume of DW equal to 1.2x the volume of the [M] 4 Mass / % volume agarose solution in a 50 mL tube.

> Note
>
> A larger volume of gelatin solution is required because some loss may occur during transfer of the viscous solution.

5 Place the [M] 10 Mass / % volume gelatin solution in the 60 °C water bath for 00:20:00 to dissolve completely.

6 While maintaining 60 °C , mix the [M] 10 Mass / % volume gelatin solution with the [M] 4 Mass / % volume agarose solution at a 1:1 volume ratio using a serological pipette.

7 Adjust the water bath temperature to 40 °C to cool the final [M] 2 Mass / % volume agarose– [M] 5 Mass / % volume gelatin solution.

Sample Preparation

8 Transfer small tissue fragments or spheroids into Microtubes and wash twice with DPBS.

9 Add > 20× sample volume of neutral buffered formalin (NBF) solution to the tube and fix at Room temperature for 00:10:00 - 00:30:00, depending on sample size.

Note

If the sample is not fixed before double embedding, it may be distorted by water imbibition.

10 Wash the sample twice with DW, each for 00:05:00.

First Embedding

11 Place the sample onto a Petri dish.

Note

Transfer the samples with a small amount of water using a pipette with the tip cut off to minimize damage.

12 Remove excess water around the sample with tissue paper.

Note

Proceed immediately to the next step before the sample dries.

13 Add 200 µL of [M] 2 Mass / % volume agarose– [M] 5 Mass / % volume gelatin solution onto the sample.

Note

Keep the area around the sample free of air bubbles.

14 Using pipet tips or forceps, position the sample at the center and adjust it so that the desired cutting direction faces downward.

15 Allow the gel to solidify at Room temperature for 00:40:00.

16 Trim the solidified gel into a pyramid shape (~25 mm³ base) using a blade.

Note

The pyramid shape helps maintain the orientation of the sample during subsequent processing.

Citation

McClelland KS, Ng ET, Bowles J (1970)
. Agarose/gelatin immobilisation of tissues or embryo segments for orientated paraffin embedding and sectioning.
Differentiation; research in biological diversity.

https://doi.org/10.1016/j.diff.2015.12.001 LINK

17 Transfer the pyramid-shaped gel into a Microtube containing 1 mL of NBF solution.

Note

Unfixed gels tend to stick together; therefore, fixation must be performed in separate Microtubes for each sample.

18 Fix at 4 °C for 24:00:00 .

19 Proceed with routine paraffin embedding according to standard procedures.

Protocol references

[1] McClelland KS, Ng ET, Bowles J. Agarose/gelatin immobilisation of tissues or embryo segments for orientated paraffin embedding and sectioning. Differentiation. 2016;91(4-5):68-71 doi:10.1016/j.diff.2015.12.001.

Citations

Step 16

McClelland KS, Ng ET, Bowles J. Agarose/gelatin immobilisation of tissues or embryo segments for orientated paraffin embedding and sectioning.
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