PROTOCOL FOR:

Agarose–gelatin double embedding prior to paraffin processing

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LEGEND

ATTENTION

HINT

REST

READEGENTS AND MATERIALS

Reagents

Chemicals/solutions	Abbreviation/Synonym	Catalog Number	Manufacturer
Agarose		75510-019	Invitrogen
Gelatin, from porcine skin	Gelatin	G1890	Sigma
DPBS		DPB 001	SolBio
Formalin solution, neutral buffered, 10%	NBF	HT501128	Sigma

Materials

Ware	Abbreviation/Synonym	Catalog Number	Manufacturer
200 µL pipet tip		T-200-Y	Corning

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

PROCEDURE

Preparation of the First Embedding Gel

- Set the water bath to 60 ℃.
- Prepare a 4% 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.
- △Keep the 4% agarose solution in the 60 ℃ water bath.
- Prepare a 10% gelatin solution: add gelatin powder to a volume of DW equal to 1.2× the volume of the 4% agarose solution in a 50 mL tube.
- ✚A larger volume of gelatin solution is required because some loss may occur during transfer of the viscous solution.
- △Place the 10% gelatin solution in the 60 ℃ water bath for 20 min to dissolve completely.
- While maintaining 60 ℃, mix the 10% gelatin solution with the 4% agarose solution at a 1:1 volume ratio using a serological pipette.
- △Adjust the water bath temperature to 40 ℃ to cool the final 2% agarose–5% gelatin solution.

Sample Preparation

- Transfer small tissue fragments or spheroids into Microtubes and wash twice with DPBS.
- △Add > 20× sample volume of neutral buffered formalin (NBF) solution to the tube and fix at room temperature for 10–30 min, depending on sample size.
- ✚If the sample is not fixed before double embedding, it may be distorted by water imbibition.
- Wash the sample twice with DW, each for 5 min.

First Embedding

- Place the sample onto a Petri dish.

- Transfer the samples with a small amount of water using a pipette with the tip cut off to minimize damage.
- Remove excess water around the sample with tissue paper.
- Proceed immediately to the next step before the sample dries.
- Add 200 µL of 2% agarose–5% gelatin solution onto the sample.
- Keep the area around the sample free of air bubbles.
- Using pipet tips or forceps, position the sample at the center and adjust it so that the desired cutting direction faces downward.
- Allow the gel to solidify at room temperature for 40 min.
- Trim the solidified gel into a pyramid shape (~25 mm^3 base) using a blade.
- The pyramid shape helps maintain the orientation of the sample during subsequent processing [1].
- Transfer the pyramid-shaped gel into an Microtube containing 1 mL of NBF solution.
- Unfixed gels tend to stick together; therefore, fixation must be performed in separate Microtubes for each sample.
- Fix at 4 ℃ for 24 h.
- Proceed with routine paraffin embedding according to standard procedures.

EQUIPMENT

Equipment	Abbreviation/Synonym	Catalog Number	Manufacturer
AX1502KR			Ohaus
MWO-18M1	Microwave oven		SK Magic
BW-10E	Water bath		Jeiotech

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.

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.