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Review article

# Agarose/gelatin immobilisation of tissues or embryo segments for orientated paraffin embedding and sectioning

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ABSTRACT

The technique described in this protocol allows the user to position small tissues in the optimal orientation for paraffin embedding and sectioning by first immobilising the tissue in an agarose/gelatin cube. This method is an adaptation of methods used for early embryos and can be used for any small tissues or embryo segments. Processing of larger tissue sections using molds to create agarose/gelatin blocks has been described previously; this detailed protocol provides a method for dealing with much smaller tissues or embryos ( $\leq 5$ mm). The tissue is briefly fixed then an agarose/gelatin drop is created to surround the tissue. The tissue can be orientated as per the user's preference in the drop before it sets as is carved into a cube with a domed top. The cube is then dehydrated and goes through the embedding and sectioning process. The domed cube is easy to orientate when embedding the tissue in a wax block giving the user assured orientation of the small tissue for sectioning. Additionally, the agarose/gelatin cube is easy to see in the unmolded wax once embedded, making the region of interest easy to identify.
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**Contents**



## 1. Materials

### 1.1. Reagents

(All reagents can be made RNAse free if required)

- Phosphate-buffered saline (PBS).
- 4% Paraformaldehyde (PFA)/PBS.
- 4% low melting agarose: $H_20$ stock.
- 5% gelatin: $H_20$ stock.
- 70% ethanol in a sealed container.

*Abbreviations:* PBS, phosphate-buffered saline; PFA, paraformaldehyde; SOX9, (sex determining region Y)-box 9; DAPI, 4′,6-diamidino-2-phenylindole nuclear stain; A/G, agarose/gelatin

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### 1.2. Equipment

- 10 mL Falcon tube (Corning).
- Laminated black cardboard/paper/stiff plastic.
- Petri dishes, 10 cm.
- Straight scalpel blades.
- Straight razor blades.
- Markers.
- Forceps.
- Pipette and tips (200 μL usually, but you may also need 1000 μL depending on tissue size).
- Mesh embedding cassettes (such as Tissue-Tek® Mesh Biopsy Cassette System; (#100500-370, VWR)) or cassettes with small pore size (such as Shandon Biopsy Processing/Embedding Cassettes; (#1001097, Thermo Scientific)).

## 2. Method

Embedding of tissues in 1.5–2% low melting agarose is successful (Blewitt et al., 1982; Buzzell, 1975; McClelland et al., 2015; Svingen et al., 2011) but a mix of agarose and gelatin (2% agarose and 2.5% gelatin, as opposed to just agarose) helps to prevent the edges of the tissue curling and wrinkling once they are mounted on glass slides (Jones and Calabresi, 2007). Protocols for larger tissues such as sections of spinal cord (Jones and Calabresi, 2007) have been published previously; this protocol is optimised for small tissues and embryo segments.

This protocol is amenable to a range of different tissue types, whether they have been freshly dissected, dissected and stored, or recovered post organ culture or post *in situ* hybridisation. If tissue has been pre-fixed proceed to Step 5. Most small tissues will require approx. 30 μL of agarose or agarose/gelatin to create a good-sized drop. Larger tissue may require up to 100 μL agarose or agarose/gelatin to create a drop that can house the tissue. The agarose/gelatin will allow tissue positioning for approximately 30–60 s.

*Preparation of stocks and solutions*

Step 1. Prepare stocks of each of 4% low melting agarose (NuSieve GTG Agarose, Lonza, #50080) and 5% gelatin in water or PBS. It is advisable to make at least 5–10 mL of each of these solutions and to store at 4 °C in Falcon tubes. Remelt when required.

a. If you are remelting agarose and gelatin it will take approx. 40 min in a 65 °C hybridisation oven or water bath to liquify.

Step 2. Using the melted stock solutions add equal quantitites to prepare 1 mL 4% low melting agarose/5% gelatin in water (final solution: 2% agarose/2.5% gelatin; in Fig. 1A–G a dye has been added to the agarose/gelatin to make it easy to observe. It is not recommended that you add dye as it will make the tissue difficult to see and orientate.)

*Preparation of tissue*

Step 3. Wash tissue twice in PBS for 5 min.

Step 4. Fix tissue in 4% PFA/PBS for 10–30 min at room temperature depending on tissue size. Approximately 10 min fixation is recommended for tissues ≤ 1.5 mm. Fixation of 10–30 min is recommended for tissues 1.5–5 mm.

Step 5. Wash tissue twice in PBS for 5 min.

*Embedding in agarose/gelatin*

Step 6. Define with a marking pen up to 10 distinct regions on a Petri dish and place on laminated black card. Using a pipette, place tissue (in PBS) in the appropriate region of the dish (Fig. 1A).

Step 7. When the agarose/gelatin mix is warm but not hot (approximately 40 °C) use a 200 μL pipette to remove as much PBS as possible from the drop surrounding the tissue (Fig. 1B).

Step 8. Add drops of warm agarose/gelatin mix over tissue until relatively thick (Fig. 1C).

a. Agarose/gelatin mix should be warm not hot; it should not create condensation on the plate.
b. Most small tissues ( ≤ 1.5 mm) will require approx. 30 μL of agarose/gelatin. Larger tissues (1.5–5 mm) may require up to 100 μL.
c. The agarose/gelatin will be fluid enough to allow tissue positioning for approximately 30–60 s.

Step 9. Once in the drop the tissue can be rearranged using forceps to the optimal orientation for sectioning (Fig. 1D). When the tissue is in position put the lid on the petri dish and leave the drop to set for 10–20 min. Do not leave for more than 40 min as the small drop will dehydrate rapidly.

a. Placing the petri dish on a black card will make orientation of the tissue much easier.
b. A dissecting scope may be used to orientate the tissue if the tissue is particularly small or translucent.

Step 10. Using a scalpel blade trim excess agarose/gelatin from the drop to form a domed cube.

a. There should be a margin of at least 0.5 mm of agarose or agarose/gelatin around the tissue (see Fig. 1E).

Step 11. Using a flat blade lift the translucent cube off the petri dish (Fig. 1F).

Step 12. Place in labelled cassette (Fig. 1G) and store in 70% ethanol for at least 24 h at room temperature (48–72 h at room temperature is recommended for larger tissues).

*Tissue processing*

Step 13. Put cassettes through a 4–6 h tissue processing dehydration run. We recommend a protocol with 30 min dehydration steps and 90–120 min in paraffin. The temperature of the paraffin in the final steps should not go above 58–60 °C as higher temperatures will melt the agarose/gelatin cube.

*Embedding*

Step 14. Embed agarose/gelatin cubes in wax

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