Paraffin embedding for mouse tissue

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Abstract

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Protocol

Sacrifice mouse

Step 1.

Sacrifice the mouse by an overdose intraperitoneal injection of sodium pentobarbital.

Sacrifice mouse

Step 2.

Fix the mouse on the tray, spray with 70% ethanol.

Tissue isolation

Step 3.

Open the mouse, remove the tissue. For aorta samples, dissect the upper half descending aorta along with heart, rinse in phosphate-buffered saline (PBS).

Tissue fixation

Step 4.

Transfer the tissue into a 15 ml tube filled with 4% paraformaldehyde (PFA) in PBS, incubate at 4°C overnight with a gentle agitation.

TEMPERATURE
4 °C Additional info:

Tissue dehydration

Step 5.

Wash in PBS twice, 5 minutes/each.

Tissue dehydration

Step 6.

Dehydrate tissue in graded series of ethanol (EtOH): 25% EtOH/PBS, 50% EtOH/PBS, 75% EtOH/dH_2O, 100% EtOH, 30 minutes each with a gentle agitation at room temperature (RT).

Tissue dehydration

Step 7.

Change the 100% EtOH with a new one, the tissue can be stored for several months at -20°C.

TEMPERATURE

-20 °C Additional info:

Tissue dehydration

Step 8.

Clear tissue twice in xylene, incubate for 1 h with a gentle agitation at RT.

Making the paraffin block

Step 9.

Incubate the tissue in 50/50 xylene/paraffin with an agitation at 60°C for 1 h.

TEMPERATURE

60 °C Additional info:

Making the paraffin block

Step 10.

Incubate the tissue in 100% paraffin, three times, 1 h each (or up to overnight) at 60ºC with an agitationn.

TEMPERATURE

60 °C Additional info:

Making the paraffin block

Step 11.

Embed the tissue.

Making the paraffin block

Step 12.

Store blocks at 4ºC until needed.