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Version 2

Lung processing and fixation for cryosectioning V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the fixation of lung tissue before cryopreservation. This protocol is required when the tissue will be used for staining intracellular proteins.

Materials

	A	B	C
	Reagent ID	Vendor	Catalog #
	Fisher Healthcare Tissue-Plus O.C.T. Compound	Fisher	23-730-571
	2-Methylbutane (Isopentane)	Millipore-Sigma	M32631-4L



PROTOCOL

7h

1

Note

NOTE: Steps 1-7 can be done in a 12 well plate.

2 Perfuse and lavage the lungs with PBS

3 Inflate the lungs with  0.75-0.9 mL of 2% PFA/50% OCT and tie the trachea

a) Be careful not to overinflate.

b) Gently lift the inflated lung and remove it with the help of scissors.

4 Place the lungs in 4% PFA (can be done in a 12 well plate –  2-3 mL)

5 Incubate  01:00:00 at room temperature rocking the plate

1h

a) This incubation can be done overnight, at  4 °C .

6 Decant PFA and rinse with PBS at least 4 hours

a) The PBS wash step can be done overnight, at  4 °C .

7 Keep the tissue at room temperature for 30-60 minutes and transfer sequentially to:

3h

a) 5% Sucrose,  00:45:00

b) 10% Sucrose,  00:45:00

c) 12.5% Sucrose,  00:45:00

d) 15% Sucrose,  00:45:00

e) 20% Sucrose--- **overnight** at  4 °C .

8 Incubate the tissue in the infiltration solution for  02:00:00 at room temp.

2h

a) INFILTRATION SOLUTION: Mix 40% Sucrose : OCT 1:1. Do not vortex the infiltration solution, as too much foam will form. Better swivel it.

b) Final concentrations of the infiltration solution: 50% OCT/ 20% Sucrose in PBS



- 9 Prepare a  500 mL beaker with around  300 mL of Isopentane or 100% Ethanol. Fill a Styrofoam box with dry ice and shove the beaker inside, to create a dry ice bath. Cap the box until the freezing step.
- 10 Transfer tissues from the plate to labelled individual plastic cryomolds. Arrange the tissue as desired and add a drop of 100% OCT (sufficient amount to just cover the tissue) and incubate the cryomold + tissue at  4 °C for  01:00:00 . 1h
- 11 Using tweezers, gently immerse just the bottom of cryomold in the dry ice bath (Beaker containing 100% ethanol or isopentane, surrounded by dry ice in a styrofoam box prepared in the step #8) and wait until the tissue piece is frozen / fixed to the mold;
- 12 Slowly add more 100% OCT on top of the frozen part, filling the cryomold up to 1 cm;
- 13 Finish freezing the entire cryomold, avoiding ethanol to get inside the mold and/or erasing the labels. If using Isopentane, you can fully immerse the mold and leave it for a while;
- 14 Transfer frozen molds to dry ice until the whole tissue is frozen
- 15 Cover in aluminum foil and put in a plastic bag or cryobox. Store at  -80 °C .
- 16 Cut and stain as desired.

Protocol references

Adapted by Italo Castro