Nov 22, 2024 Version 1

Lung processing and fixation for cryosectioning V.1

DOI
https://dx.doi.org/10.17504/protocols.io.q26g71p28gwz/v1

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Protocol Citation: Carolina Lopez 2024. Lung processing and fixation for cryosectioning . **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g71p28gwz/v1

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

Created: May 28, 2024

Last Modified: November 22, 2024

Protocol Integer ID: 100731

Keywords: fixation of lung tissue, lung processing, cryopreservation, lung tissue, staining intracellular protein, tissue, fixation, intracellular protein

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) 5% Sucrose: 20% Sucrose 2:1--------- 00:45:00

c) 5% Sucrose: 20% Sucrose 1:1--------- 00:45:00

d) 5% Sucrose: 20% Sucrose 1:2--------- 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