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Perfusion/ Fixation for cryostat slicing for HHC

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

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Abstract

This protocol details the Perfusion and Fixation for cryostat slicing for HHC.

Materials

Composition:

SUCROSE SOLUTION 30%	500 mL	1L
dH2O	250 mL	500 mL
Sodium Phosphate Monobasic (NaH2PO4-H2O)	2.691g	5.382g
Sodium Phosphate Dibasic Anhydrous (Na2HPO4)	4.331g	8.662g
Mix until salts are completely dissolved.		
Sucrose	150g	300g
Complete to final volume with dH2O, filter Adjust pH to 7.4, filter and store at 4 degrees		

Cryostat slicing

2d 1h 36m 30s

1 Weight the animal.

2 Anesthetize the animal with sodium pentobarbital (100 mg/kg et 7 mg/ml) by i.p. injection with a 27G needle.

3 Confirm the absence of pain reflex (paw pinch).

4 Perfuse the animal with 50 mL of PBS followed by 50 mL of PFA 4% by trans-cardiac injection with a butterfly needle while the right atrium is detached from the superior vena cava.

5 Extract the brain and immerse it 48:00:00 in PFA 4% followed by 48:00:00 - 96:00:00 in sucrose 30% (until the initially floating brain drops to the bottom of the tube) 2d 1h 36m

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6 Using dry ice, cool isopentane to -30 °C and submerge the brains for 00:00:15 - 00:00:30 in the freezing solution before storing them at -80 °C . 30s