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seqFISH Tissue Preservation V.3

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ABSTRACT

Tissue Preparation for seqFISH

MATERIALS TEXT

Reagent	company	Cat#
16% PFA RNase free ampules	Electron Microscopy	100503-917
10x PBS, RNase free	invitrogen	AM9625
Ultrapure water, DNase and RNase free	invitrogen	10977-015
RNAzap	Invitrogen	AM9780
Ultra pure Sucrose, RNase free	VWR	0335

Material	Company	Cat#
50 ml tube, DNase and RNase free	Corning	430290
15 ml tubes, DNase and RNase free	Corning	430052
1.7 ml microcentrifuge tubes, DNase and RNase free certified	VWR	87003-294

Fixation

- 1 Clean surface with RNAzap. Make the following solutions.

1L PBS 1x, RNase-free

1. take 1 L RNase-free water bottle
2. use serologic pipet and draw up 100 mL water into another container
3. add 100 mL of 10x RNase-free PBS
4. mix thoroughly

50% sucrose

1. tare 1 L RNase-free disposable container
2. take 500 g RNase-free sucrose container
3. add RNase-free water to sucrose and transfer to 1 L container
4. continue to add RNase-free water until mass to 1 kg
5. mix thoroughly, may need agitation on platform at RT for some time
6. store at 4°C

NOTE: for fixation, minimum PFA to tissue volume is 20:1, ideal closer to 50:1

- 2 Prepare 4% PFA in 1x PBS, 120 mL total for 4 tissues 30 mL each in 50 mL conicals.

prepare 3x conicals for 4 tissues	
16% PFA	10 ml
10x PBS	4 ml
RNase free water	26 ml
total	40 ml

- 3 Cut freshly dissected tissue to small pieces for heart, try cutting slab, 2 cm x 1 cm x 0.4 cm thickness: 2 cm dimension from epicardium to endocardium.
- 4 Place each piece in a 50 ml tube (or smaller if you can cut to smaller pieces) in 4% PFA leave at RT for 3 hrs.
** the sample should be completely covered.
- 5 Wash three times with equal volume (30mL) of 1x PBS to remove PFA leave a bit of extra liquid in the bottom between each wash.

Sucrose gradient

- 6 Place the tissue in 10% Sucrose at RT and wait for it to sink in 50 mL conical, add 10 mL of sucrose wait for sample to sink to bottom – minimum incubation time of 30 min remove supernatant for next bath.
- 7 Place the tissue in 20% Sucrose at RT and wait for it to sink.

- 8 Place the tissue in 30% sucrose in 4°C – ready for shipping. Make sure there is enough 30% sucrose solution in container e.g. if in 50 mL conicals, have 50 mL of 30% sucrose.



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