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seqFISH Tissue Preservation V.3

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Tissue Preparation for seqFISH

Materials

	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
	Ultrapure Sucrose, RNAse free	VWR	0335

	Material	Company	Cat#
	50 ml tube, DNAse and	Corning	430290

	RNAse free		
	15 ml tubes, DNAse and RNAse free	Corning	430052
	1.7 ml microcentrifuge tubes, DNAse and RNAse free certified	VWR	87003-294

Troubleshooting

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 4ºC

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 4ºC – 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.