HuBMAP Tissue Preservation Protocol v2

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1 Works for me
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1 HuBMAP Tissue Preservation Protocol Version 2

General Note: Keep collected tissues cooled on ice as much as possible to minimize warm ischemic damage prior to freezing and storage.

Flash freezing tissues

1. Place metal block into bucket of ice to cool.

2. Place disposable petri dish onto the metal block to cool; use this as the working surface to keep tissues cool as they are processed. Use a new petri dish for each tissue to avoid contamination.

3. Remove tissue from ice cooler and place onto cooled petri dish. Rapidly dissect into pieces small enough to fit into cryo tube.

4. Float aluminum foil tray in container with liquid nitrogen. Use a new foil tray for different tissues to avoid contamination.

5. Place pieces of tissue onto floating foil tray to rapidly freeze.

6. Transfer frozen pieces of tissue into labeled cryotube.

7. Place the cryotube into second container of liquid nitrogen to keep frozen while processing subsequent tissues.

8 .Record freezing time.

SeqFISH tissue preservation

Note: Keep all reagents as RNAse-free as possible

Fixation

1. Prepare 4% PFA in 1x PBS. Minimum PFA to tissue volume is 20:1; better to use 50:1.

2. Cut freshly dissected tissue to small pieces (eg 1 x 1 x 0.5 cm)

3. Place each piece in a 50 ml conical in 4% PFA and incubate for 16 - 24 hours. Place on rocker for gentle agitation.

4. Wash three times with equal volume of 1x PBS to remove PFA. Leave a bit of extra liquid in the bottom between each wash.

Sucrose incubations

5. Prepare equal volumes of 10%, 20%, 30% sucrose solutions in 50 mL conicals.

6. Place the tissue in 10% sucrose at room temperature on gentle rocker and wait for it to sink. If the tissue sinks before 30 min, leave the tissue for at least 30 min before proceeding. Some tissues that are fatty may never sink; if the tissue does not snik after 60 min incubation, proceed.

7. Place the tissue in 20% sucrose at room temperature on gentle rocker and wait for it to sink. If the tissue sinks before 30 min, leave the tissue for at least 30 min before proceeding.

8. Place the tissue in 30% sucrose at room temperature on gentle rocker and wait for it to sink. If the tissue sinks before 30 min, leave the tissue for at least 30 min before proceeding.

OCT mounting

9. Place metal block in liquid nitrogen bath to cool

10. Label a cryomold and cool on ice.

11. Place the tissue from 30% sucrose into the cryomold and slowly pour OCT over tissue with care not to form bubbles. Attempt to tease any bubbles out using a pipette tip.

12. Transfer the cryomold with tissue in OCT onto metal block cooled in liquid nitrogen. Leave in place until the OCT is frozen through (turns opaque).

13. Transfer to frozen cryomold into a chilled / labeled container. Keep on dry ice or liquid in nitrogen bath to keep frozen until transferred into -80C freezer for storage.

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OCT mounting of fresh tissues

1. Place metal block into bucket of ice to cool.
   Place second metal block in liquid nitrogen bath to cool.

2. Place disposable petri dish onto the metal block in ice to cool; use this as the working surface to keep tissues cool as they are processed. Use a new petri dish for each tissue to avoid contamination.

3. Remove tissue from ice cooler and place onto cooled petri dish. Rapidly dissect into pieces of appropriate size.

4. Label a cryomold and cool on ice.

5. Place the tissue piece into cryomold and slowly pour OCT over tissue with care not to form bubbles. Attempt to tease any bubbles out using a pipette tip.

6. Transfer the cryomold with tissue in OCT onto metal block cooled in liquid nitrogen. Leave in place until the OCT is frozen through (turns opaque).

7. Transfer to frozen cryomold into a chilled / labeled container. Keep container on dry ice or in liquid nitrogen bath to keep frozen until transferred into -80C freezer for storage.

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