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Yale Murine TMC - Tissue Preparation Protocol V.1

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Yale SenNet murine TMC

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

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Abstract

Protocol for embedding murine spleen, thymus and lymph node.

Troubleshooting

RNA Buffer (use before embedding tissue in OCT)

1 1x PBS-MC Recipe
- All prepared in DNAse RNAase EDTA free deionized water
- $MgCl_2$ (1 mM, add 1 ml of 1 M $MgCl_2$ stock solution into 1 L of 1x PBS)
- $CaCl_2$ (0.1 mM, add 2 ml of 0.1 M of $CaCl_2$ stock soution into 1 L of 1x PBS)
- 4% paraformaldehyde (PFA), optional
- 1 X Protease inhibitors (Catalog # 04693132001, Roche-cOmplete , EDTA-free Protease Inhibitor Cocktail, Tablets provided in EASYpacks)
- 1X Phosphatase inhibitor (Catalog # 4906837001; PHOSS-RO; Roche-PhosSTOP™ ; sufficient for 20-10 ml buffer preparations, suitable for tissue processing and immunoprecipitation)
- RNAse inhibitors (Invitrogen SUPERase•In RNAse Inhibitor (20 U/µL ; Catalog # AM2696)

2 Filter sterilize with 0.22 µm syringe filter. Store at 4°C before use. Use within 2 weeks.

3 Incubate tissue on ice in the buffer for 15 minutes or during the collection.

Tissue Preparation/Embedding

4 Collect tissue in PBS on ice, wash if necessary

5 Dehydration/Fixation

5.1 Incubate the tissue in 20% sucrose/PBS solution or 4% paraformaldehyde and allow it to sink to the bottom of the vial at overnight at 4°C.

6 Embedding

6.1 Place the fresh tissue in the center of the mold (Tissue-Tek Cryomold #4566) filled with OCT (Tissue Plus OCT Compound, Fisher Scientific catalog # 23-730-571). Be careful to remove any air bubbles. Remove air bubbles in OCT bottle, then squeeze out.

6.2 Orient the OCT-embedded tissue into the desired position in the mold. Carefully squeeze out more OCT on top of the tissue, again avoiding bubbles. Squeeze enough OCT out until none of the tissue remains exposed.

6.3 Freeze the tissue block on dry ice (or in cryostat).

6.4 Wrap each block in aluminum foil or place into a small plastic bag. Store the tissue block at -80° C until ready for sectioning.