

## **PROTOCOL**

### **TISSUE COLLECTION FOR IN SITU HYBRIDIZATION OF BIOPSY SAMPLES**

#### **For In Situ Hybridization:**

1. Place endoscopic biopsy samples in freshly prepared 4% Paraformaldehyde (see Appendix 1) for **2 hours at room temperature**. Mark down the time the tissue was placed in 4% Paraformaldehyde.
2. Take biopsy samples out and place them in 15% PBS/sucrose (see Appendix 2) for 1 hour.
3. Change into 15% PBS/sucrose for 1 more hour.
4. Change into 15% PBS/sucrose overnight at **4°C**.
5. Make cryostat blocks (see Appendix 3) store at -80°C.

## Appendix 1:

4% Paraformaldehyde:

Paraformaldehyde: 4 grams/100ml DEPC-treated PBS for ISH or regular PBS for fixation

1. Heat DEPC-treated PBS to 60°C on magnetic stirrer and then add Paraformaldehyde in the fumehood.
2. Maintain the temperature of the solution at 59°-60°C until Paraformaldehyde is dissolved. Do not exceed 60°C.
3. Use the same day or freeze in 50cc aliquots at -80°C until needed.

## Appendix 2:

### DEPC-TREATED PHOSPHATE BUFFERED SALINE (PBS):

	<i>2000 ml</i>
• NaCl	17.58 grams
• KH <sub>2</sub> PO <sub>4</sub>	0.544 grams
• Na <sub>2</sub> HPO <sub>4</sub> (anhydrous)	2.27 grams

NOTE: Makes up to 2 litres using double distilled water (dd H<sub>2</sub>O)

1. Add above reagents to 2 litres of ddH<sub>2</sub>O and stir the solution on a magnetic stirrer at room temperature.
2. In order to insure that the solution is RNase-free, add 2 ml of DEPC to 2000 ml of PBS. Add the DEPC in the fumehood and stir the solution for a minimum of 2 hours or overnight at setting 7.
3. Pour the PBS/DEPC solution into the PBS labeled bottles and autoclave the bottles 30 minutes. (The solution is autoclaved in the bottle in order to destroy the DEPC).
4. Allow the bottles to cool before adding the solution to the PBS tank.
5. To make 15% PBS/sucrose, add 15g sucrose (Sigma S-9378) for every 100ml of PBS and mix. **DEPC is Diethyl pyrocarbonate Sigma #D-5758.**

-Paraformaldehyde Sigma #P-6148

### **Appendix 3:**

#### **BLOCKING:**

- 1 Before beginning the actual blocking protocol, take a small beaker of isopentane and suspend it with wire or tape with a container or liquid nitrogen.
- 2 Block the tissue on a piece of card or cork (1x1 cm that will be provided) as follows:
  - A) Label the back of each card or cork with information about the tissue.
  - B) Place a layer of OCT on the card or cork to form a base on which you can put the tissue.
  - C) Orientate correctly each piece of tissue on the OCT base making sure the tissue is flat.
  - D) Generously add enough OCT to completely cover the tissue.
  - E) Carefully, with a pair of long forceps, place the OCT-covered section in the liquid nitrogen-cooled isopentane for 4-5 secs. The OCT should turn white.
  - F) If the tissue is not completely covered with OCT, repeat steps D and E.
  - G) Place each completed section in the liquid nitrogen until all the tissue sections are blocked (wrap in tin foil with information about tissue on it).
  - H) Use immediately or store the sections (wrapped in tin foil) in a well labeled container at -80°C until use.

**OCT** (Optimal Cutting Temperature) #4583 from Immucor Canada Inc.

Not sure if this # is valid with the US company in Georgia tel.800-829-2553.