

Tissue Dissection/Fixation/Embedding

*All solutions DEPC treated, cold and on ice

Remove uterus and rinse in 1X PBS

Dissect out implantation sites

Fix in 4% PFA in 1X PBS overnight at 4°C (with gentle rocking)

Rinse in 1X PBS three times for 5 min each

Incubate tissue in 10% sucrose/1X PBS at least 6 hours to overnight

Incubate tissue in 25% sucrose/1X PBS overnight

Fill cryomold with OCT compound

Blot 25% sucrose solution off tissue by touching to kimwipe

Place tissues into OCT in mold (make sure no bubbles around it)

Place at 4°C for at least 1 hour

Make sure tissue is oriented in correct way and put mold in methanol cooled down with dry ice until OCT freezes completely (I use a pyrex petri dish filled with no more than 5mm of methanol... add dry ice until methanol is very cold... keep adding dry ice as needed). It is important that the methanol not get into mold and dilute OCT...

Store at -80°C

Ship on dry ice

Solutions and Materials:

O.C.T. Compound – from Tissue Tek (#4583)

10% sucrose – 25g sucrose + 0.25g sodium azide into 250 ml (final volume) 1X PBS (DEPC), store at 4°C

25% sucrose – 62.5g sucrose + 0.25 g sodium azide into 250 ml (final volume) 1X PBS (DEPC), store at 4°C

20% PFA – 100g paraformaldehyde
1.9 ml 10N NaOH
DEPC water to 500 ml
heat to 56 degrees to dissolve (takes a while)
store in 10 ml or 50 ml aliquots at -20°C

4% PFA - dilute 20% PFA to 4% and add enough 20X PBS to make final solution 1X PBS. Make sure PBS stock solution is DEPC treated

DEPC water – add 0.5 ml DEPC to 1 L ddH₂O, shake vigorously, let sit overnight in fume hood and autoclave next day

20X PBS - 81.8g NaCl
1.85g KCl
14.4g Na₂HPO₄•2H₂O
2.03g KH₂PO₄

pH 7.5
up to 500 ml ddH₂O
add 0.5 ml DEPC and shake vigorously, leave O/N in fume hood
autoclave next day

Cryomolds – we use Tissue Tek disposable vinyl cryomolds (25mm x 20mm x 5mm)