Peter Mac Tissue Bank

OCT Embedding protocol

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Fresh tissue is transported back to laboratory as soon as possible.

Materials Required

- Isopentane (can be re-used. Store in bottle at RT)
- OCT medium. (stored at RT in 3rd draw, TB)
- Aluminium foil 'boat' made by moulding around the bottom of a thick texta pen
- Specimen label
- Yellow top universal tube
- Liquid nitrogen in short LN dewar
- Plastic beaker to sit inside LN dewar

Method

1. Fill dewar with LN
2. Pour approximately 200-300 ml isopentane into the plastic beaker.
3. Place beaker into LN. **NB**. Do not put beaker into LN before pouring in the isopentanel or the beaker will crack.
4. While isopentane is chilling, prepare tissue.
5. Form the foil 'boat'. Stick the specimen number to side of this 'boat'.
6. If tissue is too big for 'boat', divide as appropriate in the biohazard hood using a scalpel blade and a petri dish.
7. Put 2 drops of OCT into the 'boat' and place tissue on top in correct orientation for cutting.
8. Carefully pour OCT on top of tissue so that it is well covered. Do not allow any airbubbles to form.
9. Label the yellow top tube with P number and specimen number. Also place a specimen number sticker on the inside of the tube.
10. When isopentane is very cold, you can judge this by the viscosity (should be like honey), remove the beaker from the LN and hold the foil 'boat' in the isopropanol using forceps. Do not submerge.
11. The tissue block should freeze slowly from the outside in, obvious by the whitening of the OCT.
12. When completely white, place the OCT block inside the labeled yellow top tube and drop into the LN canister.
13. Store OCT blocks in –80deg freezer in the boxes marked 'OCT blocks'.
14. Note that an OCT block has been made in the database.
15. Allow the isopentane to warm up before pouring back into the recycling bottle.