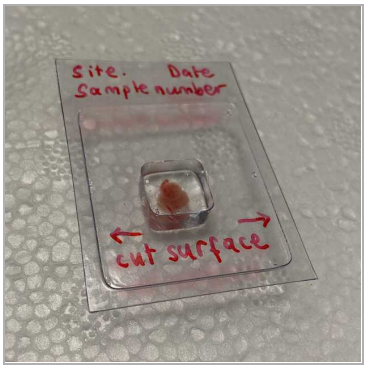


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# Sample collection, embedding and freezing tissue

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

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## DISCLAIMER

Sharp object used

## ABSTRACT

Sample collection, embedding and freezing paediatric skin tissue

## MATERIALS

Hyperthermosol - Sigma (H4416)

Isopentane - Sigma (M32631)

Cryomoulds - Agar Scientific (AGG4581)

OCT- Agar Scientific (AGR1180)

Blotting Paper – Fisher Scientific (11932985)

## SAFETY WARNINGS

This protocol uses sharp objects

## Day 1 - Sample collection

15m

1 Take 6mm punch biopsy of skin and place in a vial of Hypothermosol that has been pre-chilled at 4oC. Record time of biopsy. 5m

2 Proceed to embed and freeze the tissue as soon as possible, no longer than 24 hours later, ensuring that the sample is kept in Hypothermosol at 4oC in the mean time. Record time of freezing. 10m

## Day 1 - Embedding 26m

3 Prepare an isopentane-dry ice slurry. Place a small metal box onto dry ice, and fill with isopentane to a depth of several centimetres. Slowly add dry ice to the isopentane. 2m

4 Label cryomoulds with both sample and orientation information; it is recommended that at least the cutting face is noted on the cryomould. Half-fill cryomoulds with OCT then leave for at least several minutes; this will minimise bubbles. 2m

5 Process tissues one at a time. 10m

6 Prior to embedding and freezing, it is recommended that tissues are examined using a dissection microscope. 10m

7 Following all necessary examination and dissection, use blotting filter paper to quickly and gently remove any excess Hypothermosol. 2m

## Day 1 - Freezing 29m

8 Using spatula, transfer the tissue into a cryomould pre-filled with OCT. For skin tissue, place the sample with the epidermis facing down, and make a note of the surface needed to cut to ensure 5m

that all skin layers are sectioned

9 Carefully manipulate the orientation of the material to make sure the skin surface is at the bottom of the mould, but not touching the bottom if possible. Fill the rest of the mould with OCT so the sample is covered. 2m

10 Use a pair of forceps to place the cryomould into the isopentane-dry ice slurry, keeping it level during movement to prevent loss of OCT and sample movement. 5m

11 Rest cryomould immersed in the top of the isopentane liquid until the OCT has started to freeze 5m

12 Once completely frozen, lift the cryomould out of the isopentane-dry ice slurry, and onto fresh dry ice, to allow excess isopentane to drain and evaporate. 5m

13 Wrap each sample, frozen in individual cryomoulds, in aluminium foil. Carefully label the outside of the foil prior to wrapping around each sample. 5m

14 Transfer samples to a pre-cooled cryobox and store at -80°C. 2m