



Collection, transportation and cryopreservation of skin (dermal-epidermal) samples

Collection and transportation of samples

1. Collect samples (5 mm² or 3/8 in²) using separate, sterile instruments for each sample.
2. Place samples in screw top tubes equipped with gaskets.
3. Dilute the samples with 0.5 mL Holding Medium (recipe listed below) and ensure the tissue is fully submerged in the liquid.
4. Place the samples in a shipping cooler with multiple reusable ice packs and transport to the NAGP laboratory using an overnight courier.
5. Do not let the samples come in direct contact with the ice packs.

Cryopreservation preparation

1. For each sample, puncture both sides of a clean 0.5 mL screw-top cryovial to create holes approximately 1-2 mm (1/16 to 1/8 in) in diameter. This will enable equilibration of liquid nitrogen once the sample is submerged.
2. Label the vial with the Animal Identification number and place the uncapped vial in a rack submerged in liquid nitrogen.
3. Allow the top of the tube to be in the vapor phase of the liquid nitrogen.

Cryopreservation

1. Dilute the sample with 0.5 mL Cryopreservation Medium (recipe listed below) at 22 °C and ensure the tissue is fully submerged.
2. Incubate the samples for 5 min at 22 °C.
3. Remove the tissue from the Cryopreservation Medium and blot dry.
4. Drop the samples into the liquid nitrogen in their respective, labeled cryovials.
5. Once the liquid nitrogen stops boiling the tubes can be capped and stored in liquid nitrogen.

Note: If samples were diluted in Holding Medium for any length of time prior to cryopreservation, then make sure the sample is at room temperature (22 °C/72 °F) and remove the Holding Medium prior to Step 1 of the Cryopreservation process.

Media

Holding Medium: Minimum Essential Medium supplemented with 25 mM Hepes buffer, 0.15% (w/v) sodium bicarbonate, 100 units/mL penicillin G, 100 µg/mL gentamicin sulfate and 2 mM L-glutamine.

Cryopreservation medium: Holding medium supplemented with 2 M glycerol (14.6% by volume).

The media recipes and the collection and cryopreservation protocols are based on the manuscript:
Zieger MA, Tredget EE, Sykes BD, McGann LE. 1997. Injury and protection in split-thickness skin after
very rapid cooling and warming. Cryobiology. 35:53-69.

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