USDA ARS National Animal Germplasm Program

Skin (dermal-epidermal) Sample Collection, Transportation, Processing and Cryopreservation Protocol

Collection and transportation of samples:

Collect tissue samples (each sample is 5 mm^2 or 3/8 in^2) using separate, sterile instruments for each animal

Place up to 10 samples into a single screw top tube equipped with gaskets.

Dilute the samples with 1.0 mL Holding Medium (recipe listed below) and ensure the tissue is fully submerged in the liquid.

Place the samples in a shipping cooler with multiple reusable ice packs and transport to the NAGP laboratory using an overnight courier.

Do not let the samples come in direct contact with the ice packs.

Cryopreservation preparation:

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.

Label the vial with the Animal Identification number and place the uncapped vial in a rack submerged in liquid nitrogen.

Allow the top of the tube to be in the vapor phase of the liquid nitrogen.

Cryopreservation:

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

Place the individual samples in 0.5 mL Cryopreservation Medium (recipe listed below) at 22 °C and ensure the tissue is fully submerged.

Incubate the samples for 5 min at 22 °C.

Remove the tissue from the Cryopreservation Medium and blot dry.

Drop the samples into the liquid nitrogen in their respective, labeled cryovials. Note: only add 1 sample to each cryovial for storage.

Once the liquid nitrogen stops boiling the tubes can be capped and stored in liquid nitrogen.

Thawing:

Remove the sample from liquid nitrogen storage and place the cryovial in a 22 °C water bath for 10 min ensuring that no water contacts the sample.

Remove the sample from the cryovial and incubate the tissue in 5 ml of Minimum Essential Medium for 15 min at 22 °C.

Transfer the tissue to a separate tube containing 5 ml of Minimum Essential Medium and incubate for 5 min at 22 °C.

Process the tissue as needed for DNA extraction, cloning, etc.

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).

Reference:

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.

Versions: July 2016, April 2020