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Protocol status: In development
 We are still developing and optimizing this protocol

Skin Biopsy Protocol (Mammals): Non-lethal Sampling V.5

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Revive and Restore

ABSTRACT

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The following protocol illustrates how to collect and ship living tissue from a mammalian species under field conditions for long-term cryopreservation. Skin tissues are optimal for harvesting live fibroblast cells that can either be immediately cryobanked at -196°C, or processed for cell culture and later cryobanked at -196°C.

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GUIDELINES

Skin biopsies can be taken when animals are immobilized for veterinary exams, radio-collaring, relocation, capture and release for captive breeding, and ear tagging. The collection of samples should be opportunistic and follow all applicable regulations.

Biopsies must be **larger than 3mm³** to yield enough living fibroblasts cells for the eventual creation of cell lines. For large mammals, 5 or 6 mm biopsies are recommended. For very small mammals, 2 or 3 mm biopsies are recommended. ***Collect at least two biopsies per individual*** from different locations on the body. Collecting two samples per individual increases the chances of successful biobanking in case one sample is not successful. Only for very small mammals should one sample per individual be collected.

Biopsied tissues are stored in vials containing a nutrient-rich media to keep the cells alive and a mixture of antibiotics to prevent bacterial growth. Include only **one** biopsy sample **per vial**. If you are running low on materials, up to two biopsy can be including in one vial. **DO NOT** mix biopsies from different individuals in the same vial.

For urgent questions regarding protocol steps or collection guidelines, please contact the Revive & Restore Biobanking Team at informedbiobanking@reviverestore.org

MATERIALS

Included in Commercial Sampling Kit:

Biopsy vials with media

[Biopsy tool](#) or ear punch

- Large Mammal: 4, 5, or 6 mm Integra Biopsy Punch Dermal #33-34, 33-35, or 33-36
- Small Mammal: 2, 3, or 4 mm Integra Biopsy Punch Dermal #33-31, 33-32, or 33-34

Parafilm

Insulated Tupperware

Vinyl lunch bag

Ice packs

Styrofoam container

Biopsy form

Pre-paid FedEx shipping label

User-supplied Materials:

Sterile scalpel

Disposable gloves

Battery powered or electric clippers (optional)

Gauze

Rubbing alcohol

Disposable tweezers

Sterile scissors (optional)

[CELOX hemostatic agent](#)

Neosporin

Field notebook

Pencil/Pen

[Permanent/alcohol-resistant marker](#)

Newspaper or bubble wrap

SAFETY WARNINGS



Steps for personal safety must be considered before going into the field. Some wildlife diseases are transmissible to humans. Refer to your agency's health and safety guidelines for personal protective equipment (PPE). At a minimum, field personnel should wear disposable gloves and a fresh pair should be used between handling different specimens to avoid cross contamination. Sampling instruments and equipment should be thoroughly cleaned and disinfected or disposed of after use.

BEFORE START INSTRUCTIONS

Tissue Handling

Biopsied tissues are stored at 4°C in vials containing a nutrient-rich media to keep the cells alive and a mixture of antibiotics to prevent bacterial growth. If vials with media are not readily available, biopsies can be placed in a tube containing sterile saline and kept at 4°C. All samples must be collected under aseptic conditions to avoid contamination. Using sterile tweezers, scalpels, and scissors, as well as cleaning the sampling site with alcohol or surgical scrub (e.g. chlorhexidine) will decrease the chances of contamination. Collect as many samples as possible.

Cold Storage

Avoiding temperature fluctuations is very important for preserving the tissue samples. Use ice packs for transport of specimens from the field and ensure refrigeration is available immediately after returning from the field site. Do not use wet ice or other commercial therapeutic packs. Chill ice packs in the freezer the night before collection. Frozen ice packs will remain cold in an insulated container for up to 24 hours. If the field site is more than 24 hours from refrigeration, plan to bring a portable electric refrigerator. Vials containing tissue should not have direct contact with the ice packs. **Never freeze tissue biopsies** before shipping to a biobanking facility.

Shipping

Samples must be shipped to a biobanking laboratory via overnight express. You must use an insulated container and ice packs to maintain a temperature between 4-8°C. Considering that samples may be in queue at the receiving facility for a period of time before they are processed, **ship your samples as soon as possible**. Shipment Monday through Wednesday will guarantee arrival at the receiving facility before the weekend. **Do not ship samples on Friday**. Most facilities will not be available to receive shipments on weekends. If samples are collected Thursday through Sunday, samples must be stored in the fridge until Monday. Consult with the receiving facility for any questions regarding shipping timelines before sending samples.

Preparation

- 1 Pre-chill icepacks in the freezer the day before planning to collect and ship samples.
- 2 Record all information indicated in the biopsy form, including a picture of the animal for identification and GPS location where the animal was found.

- 3 Proper protective equipment must be worn (gloves, etc). Sterility must be maintained as much as possible.
- 4 For general sedation, anesthetize according to standard practices (refer to [Handbook of Wildlife Chemical Immobilization](#) by Terry Kreeger). For local anesthesia, use what is most appropriate for the species at hand. **Avoid use of injection or topical lidocaine.** Bupivacaine is recommended.
- 5 Shave sampling area with hair trimmer, removing as much hair as possible. Ear punches are most preferred for collection, but biopsies can also be collected from the neck, flank, or lower abdomen. Skip this shaving if the area (e.g. ear) is too small to trim.
- 6 Soak gauze with rubbing alcohol and rub shaved sampling area to thoroughly disinfect. Chlorhexidine may also be used in addition to alcohol to disinfect.

Discard used gauze. Reserve some clean gauze for use later.

Tissue Collection

- 7 Remove the biopsy vials with media from the kit and have them readily available. There are different methods for obtaining skin biopsies. The table below indicates the recommend size and number of biopsies for small and large mammals. Select the biopsy size that is safest for your species:

	Biopsy size	No. biopsies PER individual
Small Mammals	2, 3, or 4 mm	1 or more
Large Mammals	4, 5, or 6 mm	2 or more

Next, select the method below that is most appropriate for the species being handled:
Step 7 includes a Step case.

Skin biopsy tool

Ear biopsy with ear punch tool or skin biopsy tool

Sterile scalpel

step case

Skin biopsy tool

A 4mm biopsy tool is preferred. However, a 2 or 3 mm biopsy tool can be used for small mammals. A 4 mm or larger biopsy tool is ideal for large mammals. Collect ***at least 2 biopsy samples*** from different areas of the body ***per individual***. For very small mammals, one sample per individual may be collected.

Remove biopsy tool from sterile packaging. Keep the sharp metal tip sterile by avoiding contact with non-sterile surfaces. With the biopsy tool perpendicular to the skin, gently press down onto the skin in the shaved area.



Photo credit: Coyner, 2011

Use a gentle twisting motion in one direction with slight downward pressure to cut through all the layers of skin including epidermis, dermis, and the most superficial parts of the subcutaneous fat. Gently pull out the tool at a 45 degree angle, avoiding damage to the sample.

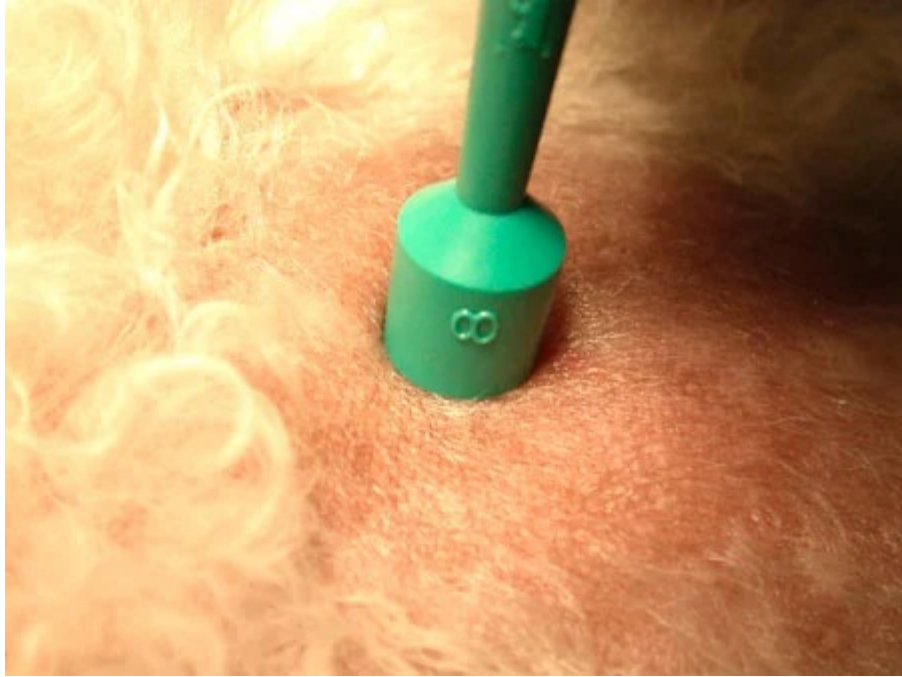


Photo credit: Coyner, 2011

Use tweezers or a 25-gauge needle to gently grasp the biopsy and pull the sample up and out. Do not crush the sample with the tweezers. If it does not lift, do not tug to tear it off. Use a razor blade or sterile scissors to cut as low as possible at the underlying fat. Discard/replace instruments between individual animals.



Photo credit: Coyner, 2011

- 8 Apply CELOX hemostatic agent to stop the bleeding and antibiotic ointment to sampling area. For larger biopsies, a suture may be needed.

Tissue Preservation

- 9 Using the disposable tweezers, immediately transfer skin piece to biopsy vial. Ensure that tissue is fully submerged in liquid. Close the cap tightly. ***Each biopsy should be placed in a separate vial.***



Photo credit: [Endangered Wolf Center](#)



Photo credit: [Endangered Wolf Center](#)

- 10** Using an alcohol-resistant marker, label the biopsy vial with an identifier that matches ***exactly*** what is indicated on the specimen form. Check to make sure that each vial is easy to identify with the information provided on the form.

Required information to include on the form:

- Scientific name of animal
- Sex of individual denoted as ♂ (male) or ♀ (female)
- Date of tissue collection
- Tissue type
- Any other identification number of individual

- 11** Add a thin layer of Parafilm around the vial covering the seal.

How to apply Parafilm:

[h](#)

- 12 Place samples on cold ice packs in an insulated cooler for transport back to your facility. Use the insulated Tupperware to transport your vials. If you do not have the Tupperware on hand, use about 3 inches of newspaper or bubble wrap to ensure that the tubes are not in direct contact with the ice packs. **Do not freeze samples.**

*****Do not ship samples via overnight express on Thursday-Sunday. For samples collected on these days, store samples upright in a refrigerator until you are ready to ship them*****

Shipping

- 13 When you are ready to ship the samples, make sure the ice packs are frozen ahead of time. Below are video instructions on how to pack your box:

[h](#)

- 14 Open the shipping box and place one frozen ice pack at the bottom of the styrofoam container inside.

Photo credit for the following photos: ViaGen Pets & Equine, 2021



- 15 Place the biopsy vials inside the insulated Tupperware container included in your kit. If you do not have the insulated Tupperware container, wrap each vial in bubble wrap or newspaper and secure with tape to avoid direct contact with the icepacks.



- 16** Close the lid and place the Tupperware container into the vinyl lunch bag provided in your kit.



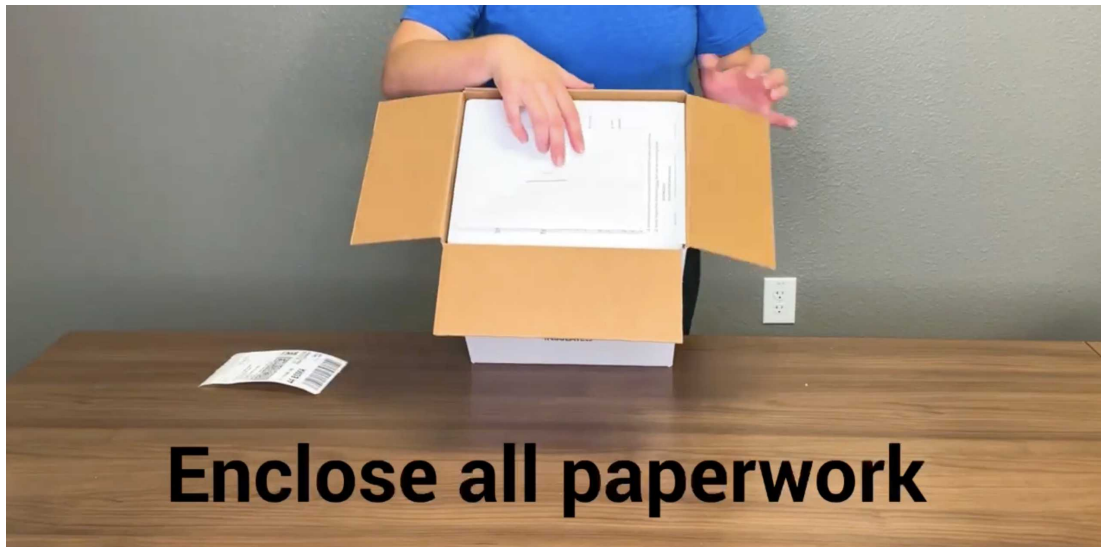
- 17** Place the vinyl lunch bag into the styrofoam container, on top of the first ice pack.



- 18** Place the second frozen icepack on top of the vinyl lunch bag.



- 19** Close the lid to the styrofoam container. Enclose all paperwork to the top, including the Biopsy Form. Close the shipping box and place the prepaid shipping label to the top.



- 20** Contact the receiving facility when you have shipped your sample and provide the tracking number so that personnel know when to expect your shipment.