

CETACEAN TISSUE BIOPSY FOR CELL CULTURE PROTOCOL

The single most important factor in taking a skin biopsy sample is the proper cleaning of the sample site. The tissue must be as sterile as possible. Bacteria or fungi kill the cell cultures. Secondary issues include time since death, ambient conditions, tissue type, and tissue condition. If the tissue is placed in the biopsy vials, the cells may remain viable for up to 2 weeks after death under some conditions, but hours or days are optimal. If biopsy vials are not available, the cells will only remain viable for 1-2 days.

Materials Needed

- ◆ 70% ethanol
- ◆ clean Q-tips
- ◆ sterile forceps (autoclaved or cleaned with 70% ethanol)
- ◆ sterile plastic weigh-boats or Petri dishes
- ◆ razor blades or scalpels
- ◆ biopsy vials (containing tissue culture media and antibiotics/antimicrobials)
- ◆ permanent marker
- ◆ Biopsy label (if possible)

Protocol

1. The most common tissue used for cell culture is skin, but additional tissues that have been successfully cultured, and offer diversity of cell types, include: Gonads, trachea (connective tissue), eye (whole, or section of retina), intestine, heart and lung.
2. Clean area with 70% ethanol to remove contaminants and (for skin) epithelial cells, use a sterile scalpel or razor blade to collect tissue and place a section (≥ 1 cm radius; more tissue is better) in clean ziplock bag or other clean, airtight container. If you have biopsy vials available, place the tissues directly in the media for transport (see transport instructions below).
3. If there will be a delay of >24 hours until delivery to the Tissue Culture facility, process the samples to further reduce contamination and improve preservation. Set out a sterile petri dish or plastic weigh-boat, a new razor blade or scalpel, and a sterilized (in ethanol) forceps. Remove the biopsy from the vial with the forceps and use a Q-tip drenched in 70% ethanol to gently clean the sample surfaces. Continue cleaning with fresh Q-tips and alcohol until the cotton tip appears free of debris/epithelial cells, and all surfaces have been wiped. Do NOT let the biopsy sit in a pool of ethanol, and rinse with a small amount of fresh biopsy media if it appears to be drying. If the tissue is larger than ~ 1 cm diameter, cut it into multiple pieces.
4. Get a fresh biopsy vial ready and loosen the cap. Be sure that the media is clear and pink. If it is cloudy or yellow, it is contaminated and cannot be used. Place the tissue for cell culture immediately into the biopsy vial and close cap tightly.
5. Using a permanent marker and label (if possible), label the biopsy vial with the species name, sex, date, animal identification number (field ID) and biopsy location (or tissue type).
6. Wrap the top of the vial and lid in ParaFilm to ensure a leak-proof seal, and store upright in a storage container.
7. The sample is now ready to ship, and should be shipped as soon as possible preferably to arrive within 24 hours. If the sample is going to be stored before shipping, keep it refrigerated (4-8°C). **DO NOT FREEZE. DO NOT SHIP FROZEN OR WITH ICE PACKS.**
8. Before sampling a second animal, sterilize the forceps in alcohol. Use a new sterile scalpel or biopsy tool. This will prevent cross contamination between samples.

Biopsy for Cell Culture Protocol

9. Biopsy vials should be protected from heat (e.g., in a cool container, either without ice packs, or insulated from ice-packs by layers of cardboard or Styrofoam to prevent contact or over-cooling). If samples are being transferred across international borders, hand carry them with CITES import and export permits USFWS CITES clearance at the border. Samples will be delivered to the SWFSC per CITES requirements, then transferred to the Beckman Center at the Safari Park.

If there are any questions regarding this protocol or if you need to request fresh biopsy vials, please call: Marlys Houck, Curator of the Frozen Zoo®, Biodiversity Banking (760) 291-5454 or the Biodiversity Banking office (760) 747-8702, x5716.

NOTE: This protocol was developed by the San Diego Zoo and adapted for conditions required for biopsy sampling of stranded cetaceans by Phil Morin (philip.morin@noaa.gov, SWFSC). Questions regarding this protocol should be directed to Marlys Houck.

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