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SIRENIAN TISSUE COLLECTION PROTOCOL FOR GENETIC ANALYSIS

Ship To: U.S. Geological Survey
Sirenia Project – Manatee Genetics Program
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Permits: Federal FWS Endangered Species Research Permit: PRT-791721
CITES Import Permit: US-808447/9

PRESERVING TISSUE WITH SED BUFFER

- Materials:**
- 1) SED buffer: saturated NaCl; 250 mM EDTA, pH 7.5; 20% DMSO
 - 2) Screw-cap plastic vials or storage containers, plastic bags
 - 3) Scalpel or clean razor blade, Cryomarker or Sharpie labeling pen
 - 4) Sterile, disposable gloves (recommended)
- Tissues:** Most tissues will suffice. Skin is readily accessible and biopsies can be easily obtained from live animals. Liver or heart muscle will also yield good DNA. Tissue samples can also be stored in ethyl alcohol or frozen for long-term storage. SED buffer, as with lysis buffer, does not require refrigeration and can have a shelf life of several years. Whole blood or spun buffy coat should be collected and stored in lysis buffer solution (blue cap tube, see separate collection protocol).
- Methods:**
- 1) Collect a few grams of tissue. A piece the size of a marble size is generally sufficient.
 - 2) Dice the tissue a few times with a clean, sharp razor blade to allow for penetration of buffer.
 - 3) Add tissue to solution in a labeled storage vial. Avoid contact with your skin and foreign objects to prevent contamination.
 - 4) Samples can be stored at room temperature or in a refrigerator for an indefinite amount of time. Avoid direct contact with sunlight or excessive heat.
 - 5) Prior to shipping, reseal tube carefully and double wrap items in plastic bags to prevent leakage.

Note 1: SED buffer is nontoxic, nonflammable, and can be stored for a long time (many years) at room temperature. Since this buffer contains salt (NaCl), you may observe a white precipitate in some tubes. This does not adversely affect the preservation ability of the buffer.

Note 2: Handling SED buffer without gloves may result in exposure to DMSO. This substance soaks into the skin very rapidly and is commonly used by physicians to alleviate muscle aches. However, DMSO will produce a vague garlic/oyster taste in the mouth along with a comparable breath odor. If you do not like this flavor, or anticipate a romantic encounter, we recommend that you wear gloves.