HUMAN TISSUE SAMPLE EXTRACTION

Background

A human tissue sample can be tested as a means of determining parentage or identity. The sample may be a product of medical examiner collection of remains, crime scene evidence collection, product of conception, or other human tissue.

Summary of Procedure

The cells in human tissue are a source of nuclear DNA. Slicing the tissue into small pieces will increase the material surface area and increase the efficiency of DNA extraction. The addition of stain extraction buffer and Pro K will release the nuclear DNA material from the cells. The nuclear DNA material can be isolated by using the organic method of extraction to remove released protein material.

Sample Handling

Forensic samples may be in limited supply. Retain sufficient sample for replicate analysis.
Label all samples with complete identifying information.

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, **Forensic Evidence Handling**.

Reagents and Materials

See **Appendix B** for reagent preparation

Stain Extraction Buffer with DTT
20mg/mL Proteinase K

Sterile transparent 2.0mL micro-centrifuge tubes and rack
Sterile transfer pipettes
Razor blade
Forceps
Disposable bench paper
Lab Coat
Gloves
Kimwipes
Permanent marker
Vortex
Biological safety cabinet
Micro-centrifuge
Weigh Boat

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Tissue Sample Extraction Worksheet** is required for documentation. All information must be completed.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions. This negative control consists of all reagents used in the procedure but contains no DNA sample.

Procedure

1. Describe the sample received in the case notes. Note the description of the sample container, specific markings, color of the tissue, specific descriptions related to preservatives added if applicable, liquid volume and any other unique identification.

2. Open the original tissue container in the biological safety cabinet with proper safety equipment. Using a sterile forceps, transfer a portion of the tissue to a sterile weigh boat. Using a sterile razor blade, slice a 2-5 mm^2 section from the sample. Slice the 2-5 mm^2 section into 1 mm pieces. Return the original sample to its container and seal the container. Place the 1mm pieces of tissue in one or more 2.0 mL sterile extraction tubes labeled with case number, sample number, analyst initials and date.

Note: The amount removed for testing is dependent upon sample type and condition. A tissue sample may require two 2.0mL tubes if degradation is suspected.

3. Add approximately 350 mL of Stain Extraction Buffer with DTT and 20 uL of Pro K to the extraction tube. These volumes may be increased if necessary. Note the volume of the above reagents added to the samples on the **Tissue Sample Extraction Worksheet**. Seal the sample tube. Vortex the mixture for several seconds to saturate the tissue with extraction buffer. Briefly pulse spin the tube to remove liquid from the lid. Place the sample in the 56°C heat block overnight (at least 16 hours).

Proceed with **Organic Extraction** starting with the addition of PCI to separate and remove unwanted proteins from the nuclear DNA material.