



THE FROZEN ARK PROJECT

Saving the DNA and viable cells of the world's endangered species

COLLECTION PROTOCOLS – BIOPSY/ TISSUE SAMPLES

Part II: Lab Protocols for Storage in the Frozen Ark

Labelling Specimens:

For long-term storage in the Frozen Ark repository, specimens should be labelled as follows:

- 1) Wherever possible specimens should be stored in appropriate tubes/containers labelled with a barcode. This is the preferred method of storage and provides a direct link between the specimens and the database. If this is not possible then tubes/containers should be labelled using a permanent marker pen on the exterior of the tube (however please note that permanent marker will wash off in ethanol and even in the absence of ethanol will rub off over time).
- 2) In addition to the exterior barcode or label, it is essential that the specimen is also labelled using a pencil (or Indian Ink marker) with the label written on waterproof paper and stored with the specimen inside the tube.

Specimen Preparation:

For large specimens take slices of tissue (minimum size 1mm x 2mm but ideally approx. 2mm x 4mm) using a sterile scalpel blade. To avoid cross-contamination ensure that a fresh scalpel blade is used for each individual.

For small specimens (where it is not feasible to take a tissue slice) store whole specimen.

Specimen Storage:

→ **Storage of Specimens upon Receipt of Live / Fresh Material:**

Where possible, for each individual specimen, all of the methods detailed below should be used for long-term preservation in the Frozen Ark repository. Equal priority should be given to each method. Where insufficient material is available to take the recommended number of tissue slices/specimens detailed below, it is preferable to distribute these equally among all methods rather than preserving them all using a single method.

Where insufficient material is available to allow all methods to be utilised (for instance, where it is only possible to obtain 1 or 2 tissue slices/specimens), we suggest preservation in the following order: **cell preservation (1), freezing (2), ethanol/ freezing (3)**. However, in countries where the electricity supply is unreliable we recommend that specimens should as a first priority be preserved in ethanol and then with alternative methods.



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1) Cell Preservation:

(4 tissue slices per specimen, where tissue slices can not be taken 4 specimens)

Preserve tissue slices/whole specimens following the tissue cell preservation protocols detailed in **Appendix 1**.

2) Freezing / Freeze Drying:

(4 tissue slices per specimen, where tissue slices can not be taken 4 specimens)

Place tissue slices/whole specimens in 2 ml screw top cryogenic/polypropylene tubes and store directly in liquid nitrogen or -80°C freezer. Where freeze drying facilities are available specimens can be freeze-dried prior to storage.

3) Ethanol / Freezing:

(4 tissue slices per specimen, where tissue slices can not be taken 4 specimens)

Preserve tissue slices/whole specimens in ethanol prior to storing in a -80°C freezer. Tissue slices/specimens should be stored in 2 ml screw top cryogenic/polypropylene tubes. To ensure adequate preservation, the ethanol should be changed a couple of times, stored overnight in the freezer and then changed again. If the ethanol remains cloudy then further changes of ethanol are required.

IMPORTANT: Ensure that pure 95%-100% ethanol is used (not IMS or alternatives).

4) Remaining Tissue:

Any remaining tissue from each specimen or additional unused specimens should be stored deep frozen (liquid nitrogen or -80°C freezer) in an appropriate cryogenic/polypropylene container.

→ Storage of Specimens upon Receipt of Frozen Material:

Where possible, for each individual specimen, all of the methods detailed below should be used for long-term preservation in the Frozen Ark repository. Equal priority should be given to each method. Where insufficient material is available to take the recommended number of tissue slices/specimens detailed below, it is preferable to distribute these equally among methods rather than preserve them all using a single method.

Where insufficient material is available to allow all methods to be utilised (for instance, where it is only possible to obtain 1 tissue slice/specimen), we suggest preservation by **freezing (1)** as a priority. However, in countries where the electricity supply is unreliable we recommend that specimens should be preserved in ethanol as a first priority.

1) Freezing / Freeze Drying:

(8 tissue slices per specimen, where tissue slices can not be taken 8 specimens)



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Place tissue slices/whole specimens in 2 ml screw top cryogenic/polypropylene tubes and store directly in liquid nitrogen or -80°C freezer. Where freeze drying facilities are available specimens can be freeze-dried prior to storage.

2) Ethanol / Freezing:

(4 tissue slices per specimen, where tissue slices can not be taken 4 specimens)

Preserve tissue slices/ whole specimens in ethanol prior to storing in a -80°C freezer. Tissue slices/specimens should be stored in 2 ml screw top cryogenic/polypropylene tubes. To ensure adequate preservation, the ethanol should be changed a couple of times, stored overnight in the freezer and then changed again. If the ethanol remains cloudy then further changes of ethanol are required.

IMPORTANT: Ensure that pure 95%-100% ethanol is used (not IMS or alternatives).

3) Remaining Tissue:

Any remaining tissue from each specimen or additional unused specimens should be stored deep frozen (liquid nitrogen or -80°C freezer) in an appropriate cryogenic/polypropylene container.

→ Storage of Specimens upon Receipt of Ethanol Preserved Material:

Where possible, for each individual specimen, all of the methods detailed below should be used for long-term preservation in the Frozen Ark repository. Equal priority should be given to each method. Where insufficient material is available to take the recommended number of tissue slices/ specimens detailed below, it is preferable to distribute these equally among methods rather than preserve them all using a single method.

Where insufficient material is available to allow all methods to be utilised (for instance, where it is only possible to obtain 1 tissue slice/specimen), we suggest preservation by **freezing (1)** as a priority. However, in countries where the electricity supply is unreliable we recommend that specimens should be preserved in ethanol as a first priority.

1) Freezing / Freeze Drying:

(8 tissue slices per specimen, where tissue slices can not be taken 8 specimens)

For freezing, drain off the ethanol prior to storage (use tissue to dry the specimen as far as possible). Place tissue slices/ whole specimens in 2 ml screw top cryogenic/polypropylene tubes and store directly in liquid nitrogen or -80°C freezer. Where freeze drying facilities are available specimens can be freeze-dried prior to storage.



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2) Ethanol / Freezing:

(4 tissue slices per specimen, where tissue slices can not be taken 4 specimens)

For ethanol freezing first replace the ethanol with fresh ethanol and then store in a -80°C freezer. Tissue slices/whole specimens should be stored in 2 ml screw top cryogenic/polypropylene tubes. To ensure adequate preservation, the ethanol should be changed a couple of times, stored overnight in the freezer and then changed again. If the ethanol remains cloudy then further changes of ethanol are required.

IMPORTANT: Ensure that pure 95%-100% ethanol is used (not IMS or alternatives).

3) Remaining Tissue:

Any remaining tissue from each specimen or additional unused specimens should be stored deep frozen (liquid nitrogen or -80°C freezer) in an appropriate cryogenic/ polypropylene container.

For further advice please contact info@frozenark.org.

Appendix 1: Tissue Cell Preservation Protocol

Requirements:

Chemicals:

Dulbecco's Modified Eagle's Medium (DMEM)

Sterile

Sigma [Cat. No. D6046 - 500ml]

Penicillin-Streptomycin-Neomycin Solution Stabilised

Sterile filtered, Endotoxin Tested, Cell culture Tested

Sigma [Cat. No. P4083 - 20ml]

Glycerol, approx 99% GC

Cell Culture Tested

Sigma [Cat. No. G2025 – 100ml]

Fetal Bovine Serum

Heat Inactivated, Sterile Filtered, Cell culture Tested

Sigma [Cat. No. F9665 – 100ml]

Equipment:

NALGENE Cryo 1°C Freezing Container – Mr Frosty [Cat. No. 5100-0001] and 250ml

Isopropanol [Sigma Cat. No. 19030 – 500ml]

Sterile Scalpel Blades



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P1000 Pipettor

P200 Pipettor

Sterile Blue Pipette Tips for P1000 Pipettor

Sterile Yellow Tips for P200 Pipettor

Sterile 2.0ml Screw Top Tube

Sterile Petri Dish (in which to dissect specimen)

Preparation of Solutions:

Antibiotics:

Aliquot a stock solution of antibiotics (Penicillin-Streptomycin-Neomycin) into sterile tubes and store at -20°C.

Slicing Medium:

For a 50ml solution, add 2.5ml antibiotics to 47.5ml DMEM. Mix well in a sterile container, and then aliquot into sterile tubes and store at -20°C.

Freezing Medium:

For a 100ml solution, add 10ml Glycerol and 20ml FBS to 70ml DMEM. Mix well in a sterile container and make 2ml aliquots in sterile tubes and store at -20°C.

Methods:

For each tissue slice (described in section A):

- 1) Cover the tissue slice in 100ul slicing medium and finely slice the tissue into tiny pieces using two sterile scalpel blades.
- 2) Transfer the sliced tissue to a sterile 2ml screw-top eppendorf tube containing 2ml freezing medium.
- 3) To freeze the cells slowly in order to preserve the cells, place the tubes inside a MrFrosty containing 250ml Isopropanol (following manufacturer's instructions) and place in a -80°C freezer overnight.
- 4) Transfer tubes to liquid nitrogen.

For further details and before sending any samples please contact the Frozen Ark office. E-mail: info@frozenark.org