

Standard operating procedure for cryo-freezing of tissue samples

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General:

- The animal tissue samples have to be collected and cryo-frozen as soon as possible in order to prevent degradation of RNA.
- Therefore, appropriate preparation of required materials and professional training of lab technicians involved in tissue sample preparation have to be performed in advance.

Materials and Preparation:

- Sterilized surgical tools and instruments and other equipment used for sample preparation and cryo-freezing (e.g., preparation plates, knives)
- Sterilized and pre-labelled (tissue type) 50 ml beakers (PFA) and petri dishes
- Pre-labelled (animal ID, tissue type, date) 2 ml cryovials (PP)
- Disposable laboratory gloves
- Spray bottles with ethanol (70%) and distilled water
- Vessels for rinsing surgical tools and instruments
- Waste buckets
- Paper towels, kim wipes
- Liquid nitrogen and appropriate cryoboxes
- Tight thermo-insulated/cryogenic gloves
- Protective glasses/goggles)

Hazard warning:

- Liquid nitrogen is a hazardous chemical. It is nontoxic but cryogenic, asphyxiant gas that can reduce the normal oxygen concentration in breathing air. Exposure to oxygen-deficient atmosphere may cause dizziness, drowsiness, nausea, diminished mental alertness, loss of consciousness and suffocation. Contact of liquid nitrogen with skin and eyes can cause severe cryogenic burns.
- Anyone handling cryogenic liquids and asphyxiant gas should be familiar with the hazards presented by such materials and trained in how to handle them safely. Liquid nitrogen should be used in a well ventilated area. To prevent personal hazards due to cryogenic liquids users have to wear personal protective equipment.

Protokoll for tissue sample collection, preparation and cryo-freezing

- The fed animals are killed according to regular slaughtering protocol including stunning and exsanguination by expert staff in an experimental slaughterhouse of the institute.
- A specific animal dissection protocol is applied to enable rapid tissue sample collection.
- Tissue samples are collected immediately (30 min) after slaughter by a veterinarian and distributed to trained lab technicians for further sample preparation.
- Preparation and cryo-freezing of tissues samples is taking place in a separate laboratory adjacently located to the slaughtering facility. The preparation team consists of 3 - 4 persons depending on the number of tissues to be sampled and collected.
- The tissue samples are meticulously dissected and cut in small cubes (2-3 mm each edge length) using surgical instruments and immediately snap-frozen in pre-labelled 50 ml beakers (PFA) containing liquid nitrogen. The beakers are located in an appropriate cryobox, also containing liquid nitrogen.
- Snap-frozen sample pieces are separately picked from the beakers and transferred into 2 - 4 pre-labelled and pre-cooled 2 ml cryovials (PP) and stored in liquid nitrogen.
- Upon completion of sample collection and preparation the cryovials are transferred to a deep freezer (-80°C) and stored there until use.

The experimental procedures were carried out according to the animal care guidelines with respect to welfare and health of the animals and were approved by the relevant authorities of the State Department of Agriculture, Food Security and Fisheries, Mecklenburg-Western Pomerania, Germany.