GeneLab Standard Operating Procedure: Frozen Tissue Cutting

May 2020

Version 1.0

Document Revisions

Document Number	Revision Number	Date	Description of Changes
GL-SOP-1.2	1.0	May 2020	Original document

Scope and Purpose

The procedure below describes the steps required to safely section a tissue prior the extraction of nucleic acids. The procedure, if followed correctly will allow portioning a piece of tissue without thawing and compromising the original biological sample.

Equipment and Consumables

1. Styrofoam box and dissection tools (forceps, scalpel, scissors)
2. Sterile Microcentrifuge Tube 1.5 mL (RINO®) or 10ml round bottom Falcon Tubes (Used for tissue homogenization, NextAdvanced Cat#TUBE1R5-S)
3. Kimwipes (Fisher Scientific, Cat#06-666 or similar)
4. Analytical balance
5. Small (2”) weigh boat (VWR, Cat#10770-454 or similar)
6. Ice bucket

Reagents

1. Wet ice
2. Dry Ice (2lb)
3. Lysis Buffer depending on the protocol used:
    a. If following SOP #3.1, on the day of tissue cutting/extraction prepare 800-800uL of QIAGEN buffer RLT + 1% b-ME per sample.
    b. If following SOP #3.2 on the day of tissue cutting/extraction prepare 600-800uL of TRIzol solution per sample.
    c. If following SOP #3.3 on the day of the tissue cutting/extraction prepare 1000uL of CTAB buffer per sample.

4. RNaseZap RNase decontamination solution (Thermo Fisher Scientific, Cat#AM9780 or Cat#AM9782 or Cat#AM9784 or similar)

Procedure

1. Fill Styrofoam box with dry ice.
2. Prepare appropriate lysis buffer.
3. Label the 1.5mL/10mL homogenization tubes. Fill the tubes with appropriate lysis buffer and keep on wet ice.
4. Sterilize dissection tools, weigh boats, bench surface and gloves with RNaseZap. Once dry, place the dissection tools in the Styrofoam box to chill.
5. In Styrofoam box with dry ice, transfer tissues from -80°C to the lab.
    a. If the tissue is RNAlater-preserved, thaw on wet ice.
    b. LN2-preserved tissues should be kept inside the dry ice box at all times.
6. Follow Table 1 and select minimum weight needed for each type of mouse tissue.

Table 1: Validated minimum weight for different tissue type.

Tissue	Minimum weight (mg) to cut
Liver	20-30
Thymus	Up to 20
Colon	20-40
Lung	20-30
Skin	40-50
Spleen	Up to 20
Muscle	Up to 20
Kidney	Up to 20
Brain	20-25
Heart	Up to 20
Lymph Nodes	10-15
Adipose Tissue	30-40
Brown Adipose Tissue	30-40
Reproductive Tract	20-25
Adrenal Gland	One adrenal

7. For both RNAlater-preserved tissues and LN2-preserved tissues, use pre-chilled dissection tools to cut tissue in the weigh boats placed on wet ice(for RNAlater tissues) or dry ice (For LN2

tissues). Return remaining tissue back to sample tube. For LN2 tissues take extra care to use cold tools and keep tubes in the dry ice container at all times.

8. Weigh each cut tissue on the analytical balance by quickly transferring the tissue from the weight boat on dry/wet ice on to a weight boat on the analytical balance. As soon as the reading stabilizes, move the tissue in the weight boat on to the dry/wet ice.
    a. If the 1st cut doesn't generate enough tissue, repeat step 7 and cut another piece from the original tissue.
9. Place the cut tissue inside designated homogenization tube and record the weight.
    a. Be extremely careful to not thaw the frozen tissues prior to submersion into lysis buffer.
10. Return the homogenization tubes to wet ice.
11. Clean dissection tools with RNAseZap before proceeding to the next tissue.
12. Once finished with cutting all tissues, return sample tubes back to -80°C storage.
13. Immediately proceed with homogenization SOP #2.1 or #2.2 and RNA/DNA extractions SOP's#3.1 or #3.2 or #3.3.
14. Clean tools with detergent and let dry on a paper towel. Keep scissors in open position while drying to avoid corrosion.