Effective date: 2022-05-26

EXTRACTION SERVICES

User Guide

RNA Extraction

Version 01

Table of Contents

General Information

This document describes the procedure to follow when requesting RNA extraction. The detailed instructions for the preparation, the samples submission, the shipping requirements, as well as any additional information are all provided in this guide.

It is best to contact the Client Management Office prior to collecting samples to receive relevant project-specific recommendations.

RNA is extremely sensitive to degradation and the quality of the extraction relies heavily on the pre-extraction handling. See the Additional Information section to obtain more information on the handling, sampling, and storage of RNA.

Specific instructions must be followed for FFPE samples. See the Additional Information section, FFPE samples for RNA extraction.

Sample Preparation

Tubes

For RNA extraction the following microtubes are accepted, only the first option (RB tubes) will not require additional costs if there is a transfer for the procedure.

- First choice:
  RB tubes for samples, 2 mL, Qiagen, Cat# 990381

- Second choice:
  Boil-proof microcentrifuge tubes, 2 mL, clear, Progene, Cat# 87-B200-C

- Third choice:
  Microcentrifuge tubes, 1.5 mL, certified free of RNase, DNase, DNA and PCR inhibitors, Progene, Cat# 87-B150-C

Requirements

Sample Volume and Quantity

Type of material	Storage Quantity[1] and temperature	Comments
Animal Tissue	50 mg in 900 µL TRIzol[2], -80°C	When making the initial request, specify the species and the type of tissue because the recommendations for sampling may differ.
Blood		Contact the Client Management Office for more information.
Animal cells (pellets)	1 to 10 million in 900 µL TRIzol[2], -80°C	All treatments (for ex. FACS) will affect the results of the extraction. Contact the Client Management Office for more information.
Plant Tissues	50 mg, -80°C	When making the initial request, specify the species and the type of tissue because the recommendations for sampling may differ.

[1] The use of more or less material than the recommended amount (+/- 10 %) will affect the quality of the extraction; respecting the ratios of the material/chemistry of the kit and the capacity of the filters used during the extraction is critical.
[2] Follow the suppliers' recommendations for optimal reagent use.

Identification

The samples are identified with the name provided by the client and a unique barcode provided by Genome Quebec.

Sample names

The samples names must be easy to write and read and must not exceed 5 characters (exp. L1, L2, L3, L4).

If the sample names are not legible or are confusing (exp. Multiple barcodes on the same tube), the samples may be returned with charge to be identified.

Each name must be unique. Do not use the same name for 2 tubes even if they are duplicates.

Le name indicated on the tube must correspond to the one submitted in the Sample Submission form.

The use of a printer for microtube labels with freezer-resistant labels is preferred over being handwritten.

The labels must be placed from top to bottom on the tube and not around it, it must not contain wrinkles, or be obstructed in any such way as to interfere with scanning. The name of the sample can be written with letters, numbers, in 1D or 2D code. See Figure 1 for an example of the orientation for the label.

Figure 1 – Example of the label identification for a sample submitted to Extraction services

In the case where access to a printer is not possible, use a fine point dark permanent marker. The name must be on both the cap and on the side of the microtube.

Barcodes: for submissions of less than 48 samples

In the sample submission of the request write the name of the samples in the column "Sample Name." The column "barcode" must stay empty. Barcodes will be added to the sample tubes by a Genome Quebec technician at the reception.

Barcodes: for submissions of more than 48 samples

Barcodes are provided by Genome Quebec. It is required to put them on the tubes before sending them.

Contact the Client Management Office to obtain the number of labels needed.

The barcode is a 1D code, scannable and legible. The number is incremental.

Here are the recommendations to follow when placing the barcode on the microtube:

1. Place the microtubes on ice. It is recommended to work with a small number of samples at a time to avoid long periods of thawing.
2. Dry the microtubes with a dry tissue, for example a Kimwipe and place the label as described above.
3. Verify the association between the barcode and sample name. If there is a mismatch during the identification of the tubes, the identity of the sample will be linked to the barcode on the tube.

Service Request Form and Sample Submission

All service request forms, and sample submissions must be done on the web through Nanuq by using a user's account. To get an account contact the Client Management Office.

Work in the laboratory will only start once all the documentation is submitted. An incomplete documentation will cause delays.

Service Request Form

1. Open a session in Nanuq.

2. Click on "Add new request" in the section "Request" and follow the instructions.

   The option "new request" does not need to be used to complete an already existing request.

   Do not use the "Back" button in your browser to go back to the previous pages. Use the menu on the left to navigate through the form.

   Click on "Next" to go to the next page of the request.

   It is always possible to save the information by clicking "Save and continue later." The drafts are accessible through "My request lists" in the section "Request." The request will stay in draft until it gets submitted. To modify a request in draft, click on "Modify" in the menu on the left.

   To request the return of samples once the project is completed, indicate it under the "Sample Information" tab and complete the requested information.

3. You must click on "Submit" so that your request can be approved by the Client Management Office. Requests that are not submitted will not be processed.

Sample Submission

Once the service request is complete and submitted, submit the samples.

1. Open a session in Nanuq.

2. If applicable, find the request using "My request list" and click to open it.

3. Click on the tab "Sample submission," and then on "Add new samples."

4. Follow the instructions on the screen.

5. Verify that the status of the submission is at "Submitted" under the "Sample submissions" tab in the Service request.

Follow the same steps to add new samples to the request or to add replacement samples.

Sample Shipment Preparation

Waybill

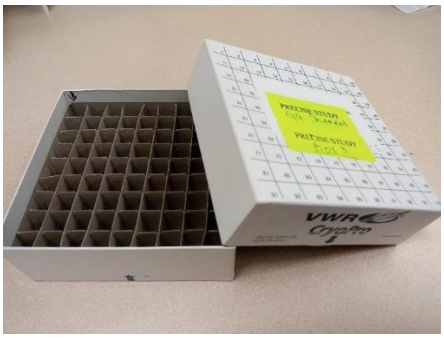
After the sample submission, return to the tab "Sample submission," select the sample submission(s) related to the package being prepared, and click on "Print waybill." By default, only one copy will print, but two are required.

Package Preparation

Do not send « floating » tubes directly in the dry ice or just in a plastic bag. This could result in tubes breaking, labels unsticking, or tubes getting lost.

Send the tubes containing the samples in a cardboard box having dividers resulting in a 9 by 9 or 10 by 10 format, see Image 2.

Image 2 – Example of a box to use for sending microtubes



Identify the cardboard box as follows: the name of the principal investigator, the shipment date and the name of the project indicated on the Request Form.

Place the tubes in order from left to right by following the same order as in the Sample Submission form.

The box must be held shut with an elastic or laboratory tape.

The boxes of samples must be sent in dry ice. If the package contains heavy items that can damage the contents during transportation (ex.: block of dry ice) it is recommended to protect it from those impacts.

The package must contain enough dry ice to keep the samples frozen until they arrive at their destination.

One copy of the waybill must accompany the samples. Make sure that the waybill stays dry by placing it in a sealed plastic bag (type of Ziploc).

Samples crossing the Canadian border should be sent at the beginning of the week to avoid the risk of them being stored at the carrier's warehouse over the weekend. The use of clear phrases such as: "non-biohazardous biological samples," "Purified DNA from [species]," "For research use only," and "No commercial value" on the commercial invoice will help expedite customs clearance.

Samples Shipment

The delivery address and the directives concerning the delivery will be found on the waybill.

One copy of the waybill must be visible on the outside of the package. It can be taped directly to the package or placed in a transparent envelope.

For More Information

Client Management Office

Telephone: 514-398-7211
Email: infoservices@genomequebec.com

Version History

Version	Summary of modifications	Author	Effective date (aaaa-mm-jj)
01	First Version	S. Power	2022-05-26

Additional Information

RNA Manipulation Instructions

RNA easily degrades and it is important to follow best practices in the laboratory including sampling and storage.

The technician must wear a clean laboratory coat (only used for RNA handling and washed often) and regularly change gloves especially when handling material that may have RNases.

It is preferable to reserve a part of the bench for RNA work. The surfaces, the pipettes, the tube supports, etc. must be washed regularly with products like TriGene or RNaseZap™ or with 70% ethanol.

All the consumables and reagents (plastic tubes, tips, water, ethanol, etc.) must be certified "RNAse-free." The tips must have filters. If certification is not possible (ex. Gloves) use a new box, found in a specific spot, and identified as exclusively used for RNA.

Factors preceding the RNA extraction and affecting the yield (quantity and quality) are:

- RNAse-free environment
- Type of material being extracted (ex. Animal/plants, pancreas/heart)
- Following the protocol
- Sample quality and storage
- Following the recommendations for the format, volume and/or quantity of material and shipment

When extracting RNA, there will be variability and in the case of samples with compromised qualities, it is possible that the collected RNA cannot be used for subsequent use.

Sampling and Storage

It is preferable to freeze the exact quantity requested in this guide to avoid a freeze/thaw cycle during a subsequent weighing and/or additional costs if the weighing is done by extraction services.

It is important to freeze the tubes in an upright position (the cap towards the top) so that if there is liquid (TRIzol), it does not freeze while pushing against the cap creating an opening.

Two methods are recommended for the preparation and conservation of the samples based on the type of material. Contact the Client Management Office to know the appropriate method to use.

- Snap-freeze method
  Dip the tubes with the material in liquid nitrogen (upright position). Once frozen, transfer the tubes to -80°C.

- TRIzol method
  Follow the recommendation given by the supplier but use 900 µL instead of 1000 µL. https://www.thermofisher.com/order/catalog/product/15596026
  At the stopping point, put the samples (upright position) at -80°C.
  The tissue must be entirely submerged in the TRIzol.

It is not permitted to send material kept in ethanol. Extraction services do not offer ethanol wash and/or evaporation services before extraction. These steps must be done before sending the material. All traces of ethanol will affect the extraction yield and will have subsequent impacts of the following steps. If such a preservation buffer is used, the results are not guaranteed.

FFPE samples for RNA extraction

The nature of FFPE samples pose additional challenges during extraction. The success rate for DNA extraction is generally high, whereas for RNA extraction the success rate could be between 50-80% depending on the criteria established for downstream analysis.

Moreover, the success rate depends on the conditions and the temperature of preservation of the material as well as adherence to the recommendations provided by the extraction platform.

Send FFPE samples at room temperature (without dry ice or other).

All other recommendations concerning sample identification and shipping must be respected.

If the material is of human nature, a positive control will be added to every 23 samples resulting in a total of 24 extractions. This serves to compare the quality of the extraction versus the quality of the samples. The extraction of the control will be charged.

A minimum number of samples may be required (charged).

Quantity[1] and storage temperature	**Format[2]**	**Comments**
Slices with an area of 150 mm^2, either 2 sections of 20 um of thickness or 4 sections of 10 um of thickness.	RB tubes for samples, 2 mL (Qiagen #990381)	Do not send material that has come into contact with the air.

[1] The use of more or less material than the recommended amount (+/- 10%) will affect the quality of the extraction, respecting the ratios of the material/chemistry of the kit and the capacity of the filters used during the extraction is critical.
[2] No other format of microtube will be accepted since the extraction starts directly in the tube and no transfer will be done.