



## SERVICE GUIDE

# RNA Extraction Service

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# Service Guide:

## RNA Extraction Service



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# Service Guide: RNA Extraction Service



## 1.0 Overview

AGRF's RNA Extraction Service provides RNA for entry into a wide range of services.

All RNA Extractions are currently performed using the PAXgene Blood RNA Kit (IVD) coupled with the PAXgene Blood RNA Tube used for Human Blood samples and the RNeasy Mini Kit for animal cells and animal tissues.

RNA yields and average molecular weights are highly dependent on the sample type and quality. Recovery of RNA yield and molecular weight suitable for intended downstream purposes are not guaranteed by AGRF.

AGRF requests that you retain a working aliquot of all samples to mitigate the unlikely risk of sample loss during transport or processing. In the event of accidental sample loss during processing, AGRF's liability will be limited to a full refund of the price of sample processing.

List of sample types for this service:

- Human blood
- Animal cells
- Animal tissues

**Note:** AGRF does not have an RNA extraction service for plant material or other types of samples not listed here.

## 2.0 Sample Submission Requirements

RNA stabilisation is an absolute prerequisite for reliable gene expression analysis. Immediate stabilisation of RNA in biological samples is necessary because, directly after harvesting the samples, changes in the gene expression pattern occur due to specific and non-specific RNA degradation as well as to transcriptional induction. Such changes need to be avoided for all reliable quantitative gene expression analyses.

The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing.

RNA in animal tissues is not protected after harvesting until the sample is treated with a stabilisation reagent, flash-frozen, or disrupted and homogenised in the presence of RNase-inhibiting or denaturing reagents. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at -90 to -65°C, or immediately immersed in a stabilisation reagent and stored according to the manufacturer's instructions.

Maximum amount of starting material:

- Animal cells: 1 x 10<sup>7</sup> cultured cells
- Animal tissues: 10-25mg fluid stabilised tissue or frozen tissue

All animal tissue samples must be pre-sized by the client to between 10-25mg (**note: amount of tissue should be written on the tube or submission sheet**) and all animal cell samples need to be pelleted by the client e.g. pellet cells at 2,000 x g for 5 minutes. Wash cells once with phosphate buffered saline to remove the culture medium. Pellet the cells again at 2000 x g for 5 minutes. Number of cells should be written on the tube or submission sheet.

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All samples should be sent in individual tubes and the maximum amount of starting material must not be exceeded. If this is exceeded, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy spin column membrane, resulting in lower RNA yield and purity.

If samples are frozen, they must not be allowed to thaw before extraction. Letting your samples go through a freeze/thaw cycle will degrade the RNA. Please keep this in mind when you pre-size your samples for extraction.

RNA in harvested animal tissue is not protected until the tissue is completely submerged in enough volume of stabilisation reagent. After harvesting, the tissue should be immediately placed in at least 10 volumes of the reagent (or approximately 10µl reagent per 1mg tissue). Larger volumes can be used if necessary or desired. Smaller volumes may lead to RNA degradation during storage. Storage containers should be wide enough so that the reagent covers the entire tissue. Storage containers or tubes with large diameters may require more reagent to completely cover the tissue. The procedures for tissue harvesting and RNA stabilisation should be carried out as quickly as possible.

Please read the Qiagen RNeasy Protect<sup>®</sup> Tissue Reagent handbook found [here](#).

Allprotect Tissue Reagent can be used for immediate stabilisation of DNA, RNA, and protein in tissues. Read more on the Qiagen website [here](#).

Invitrogen RNAlater Stabilization Solution is an aqueous tissue storage reagent that rapidly permeates tissues to stabilise and protect cellular RNA. For more information from the ThermoFisher website, click [here](#).

**NOTE: Only fresh, unfrozen tissues can be stabilised using RNeasy Protect<sup>®</sup> Tissue Reagent, Allprotect Tissue Reagent and RNAlater Stabilization Solution. Previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation. Thawing the tissue first before placing in the stabilisation fluid will also cause degradation of the RNA.**

**NOTE: For RNA in animal cells use RNeasy Protect<sup>®</sup> Cell Reagent (for RNA/DNA stabilisation).** Read more about this from Qiagen [here](#).

For human blood samples you should use the [PAXgene<sup>®</sup> Systems](#) making sure you follow the manufacturer's instructions carefully to ensure good quality RNA. The PAXgene Blood RNA Tube contains an additive that stabilises the *in vivo* gene transcription profile by reducing *in vitro* RNA degradation and minimising gene induction. For more information see the PAXgene Blood RNA tube or the PAXgene Blood RNA Kit Handbook [here](#).

### 3.0 Turnaround Time

Turnaround times for RNA extraction services are affected by service demand and sample type at the time of sample receipt. Please ask your Account Manager for a more accurate turnaround time at the time of submission.

### 4.0 Sample Storage

The RNA will not be stored at AGRF. All RNA will be sent for downstream processing. If you require your samples to be returned to you post-processing, please let your Account Manager know at the time of quoting. Please note that a fee will be charged for return of samples. Raw sample material will not be stored at AGRF. All sample material will be extracted or discarded after Quality Control.

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### 5.0 Shipping Your Samples for Extraction

The responsibility for ensuring samples reach AGRF in good condition remains with the client. If samples arrive in poor condition, we will notify you as soon as the samples arrive.

For domestic shipments (within Australia), we recommend you use a courier and ship samples on ice/cold for an overnight shipment. If your samples are already frozen, they should stay frozen and be shipped on dry ice.

**Table 1: RNA sample types and stabilisation/collection details.**

Sample Type	RNA Stabilisation/Collection	Stability
<b>Animal Tissues</b>	RNAlater RNA Stabilization Reagent (for RNA stabilisation)	RNAlater-Stabilized samples: 7 days at 15-25°C, 4 weeks at 2-8°C, Longer periods at -20 to -80°C
	RNAprotect® Tissue Reagent (for DNA/RNA/protein stabilisation) cat. no. 76104. Read more <a href="#">here</a> .	1 day at 37°C, 7 days at 18-25°C, or 4 weeks at 2-8°C, -20°C or -80°C for archival storage
	Allprotect Tissue Reagent (used for immediate stabilisation of DNA, RNA, and protein in tissues). Find out more <a href="#">here</a> .	15-25°C for 7 days or stored at 2-8°C for up to 12 months. For longer storage, stabilised tissues can be archived at -20°C or -80°C.
<b>Animal Cells</b>	RNAprotect® Cell Reagent (for RNA/DNA stabilisation) cat. no. 76526. Read more <a href="#">here</a> .	Cells stable at 30°C (1 day), 15-25°C (7 days), or 2-8°C (4 weeks). Archiving of cells at -20°C or -80°C
<b>Human Blood</b>	Human blood (stabilised in PAXgene Blood RNA tubes) PAXgene Blood RNA Tubes cat. no. 76216. Read more <a href="#">here</a> .	Up to 3 days at room temperature (15-25°C). Up to 5 days at 2-8°C, 11 years* at -20°C or -70°C

### 6.0 Online Sample Submission

Online Submission:

- Submit your sample details online.
- Select: “Extraction and (ongoing service)” as the Service Type, if the RNA is going to be moved into another AGRF service.
- Select: “Extraction” as the Service Type, if the RNA is not going to be moved to an ongoing service.
- Please complete and upload the “Template File” excel template.
- Send your samples to the address below.

#### Physical address (courier) and postal address (mail):

AGRF Ltd  
 PLANT GENOMICS CENTRE  
 WAITE CAMPUS  
 HARTLEY GROVE  
 URRBRAE SA 5064