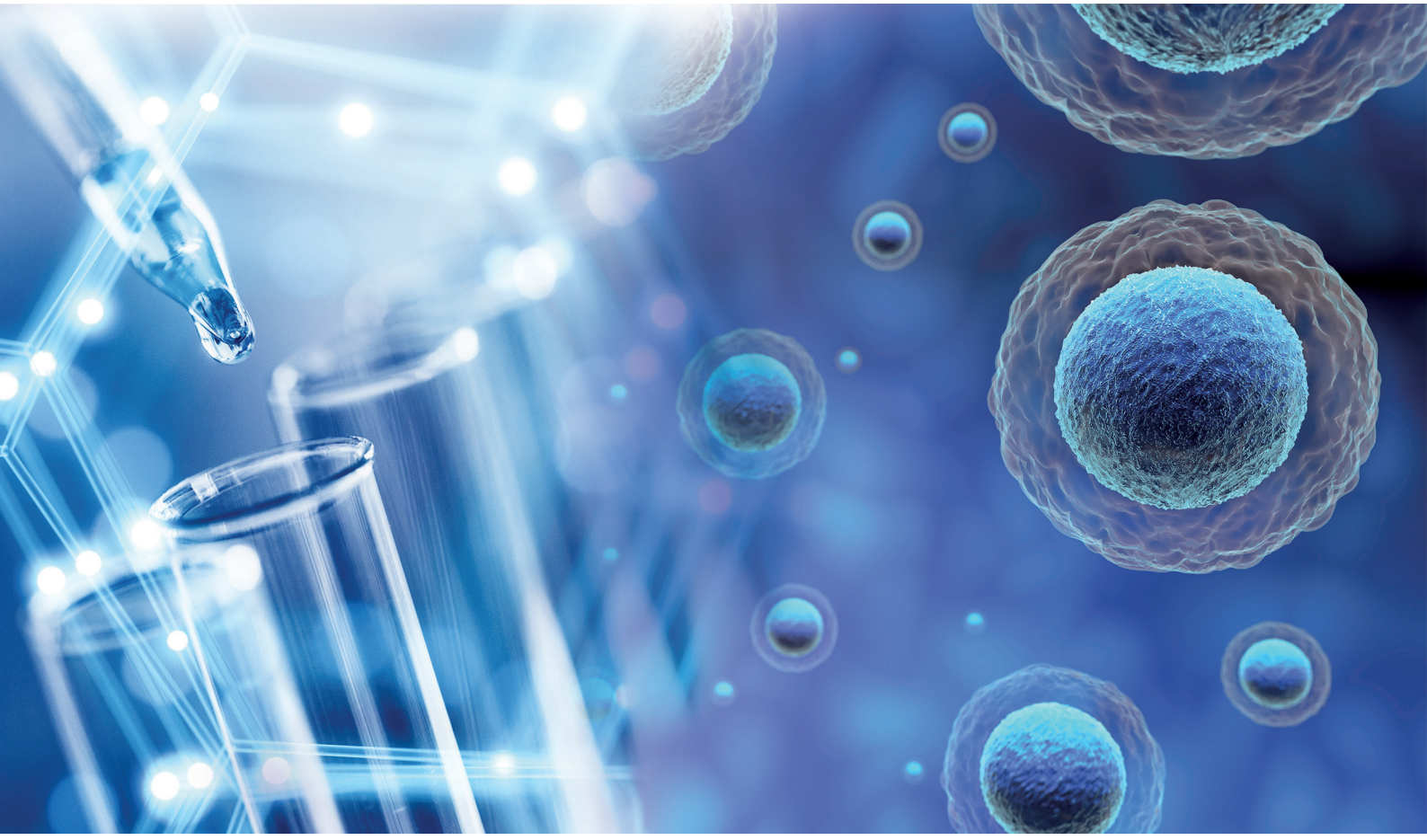
Chromatrap

# RNA Extraction Kit

## The Complete Kit for Rapid Extraction of Total RNA

Catalogue No. 500335



The Chromatrap® RNA Extraction kit is for research purposes only.
Not intended for diagnostic use.

# CONTENTS


Introduction 3
Kit Contents 5
Additional Materials 6

Protocol 7
Section 1: Sample Collection and Cell Lysis 7
1.1 Cultured Cells 7
1.1.1 Adherent Cells 7
1.1.2 Suspension Cells 8
1.1.3 Frozen Cell Pellets 8
1.2 Tissue 8
1.2.1 Soft Tissue 9
1.2.2 Frozen or Fibrous Tissue 9
1.3 Blood 9
1.4 Buccal Swabs 10
1.5 Plants 10
1.6 Yeasts 11
1.7 Bacteria 13
Section 2 | Extraction and Purification of Total RNA 14

Additional Protocols
RNA Clean Up 15
DNase Digestion 15

Troubleshooting and FAQ 16
Appendix 1: Chromatrap® Homogeniser Spin Column Protocol 17
Other Chromatrap® Products 18

# Introduction

The Chromatrap® RNA Extraction kit is a simple, fast and efficient method for extraction of high quality total RNA from cell lines, tissue, blood, swabs, yeast and bacteria. Cells are lysed and captured by a high yield membrane contained within a spin column to maximise RNA capture and minimise sample loss. Unwanted impurities are removed from the column by 2 simple wash steps leaving only purified application-ready RNA which is subsequently eluted by centrifugation (see Figure 1).

Purify up to 1,000 µg ready-to-use RNA per column suitable for downstream applications including; cDNA synthesis, RT-PCR (reverse transcription PCR) and RT-qPCR (Real Time Quantitative PCR), RNA-seq, microarrays, Northern blotting, RNAse/S1 nuclease protection, Poly(A) selection, transfection, localisation studies and primer extension.

## Samples Sources

- Animal cells, blood & soft tissue
- Plant cells
- Yeast
- Gram negative bacteria
- Buffy coat
- Buccal swabs

## Storage Buffer Compatibility

Suitable for use with samples stored in preservation reagents such as DNA/RNA Shield™, RNAprotect®, Allprotect®, Universal transport medium/viral transport medium (UTM®/VTM®) and RNAlater™.

## Advantages of Chromatrap® RNA Extraction kit

- Obtain up to 1000 µg pure RNA
- Extract application-ready Total RNA in as little as 10 mins
- Process up to; $10^7$ cultured animal cells, 50 mg animal tissue, 0.5 ml blood, 250 mg plant material, 5 x $10^7$ yeasts or 5 x $10^8$ bacteria

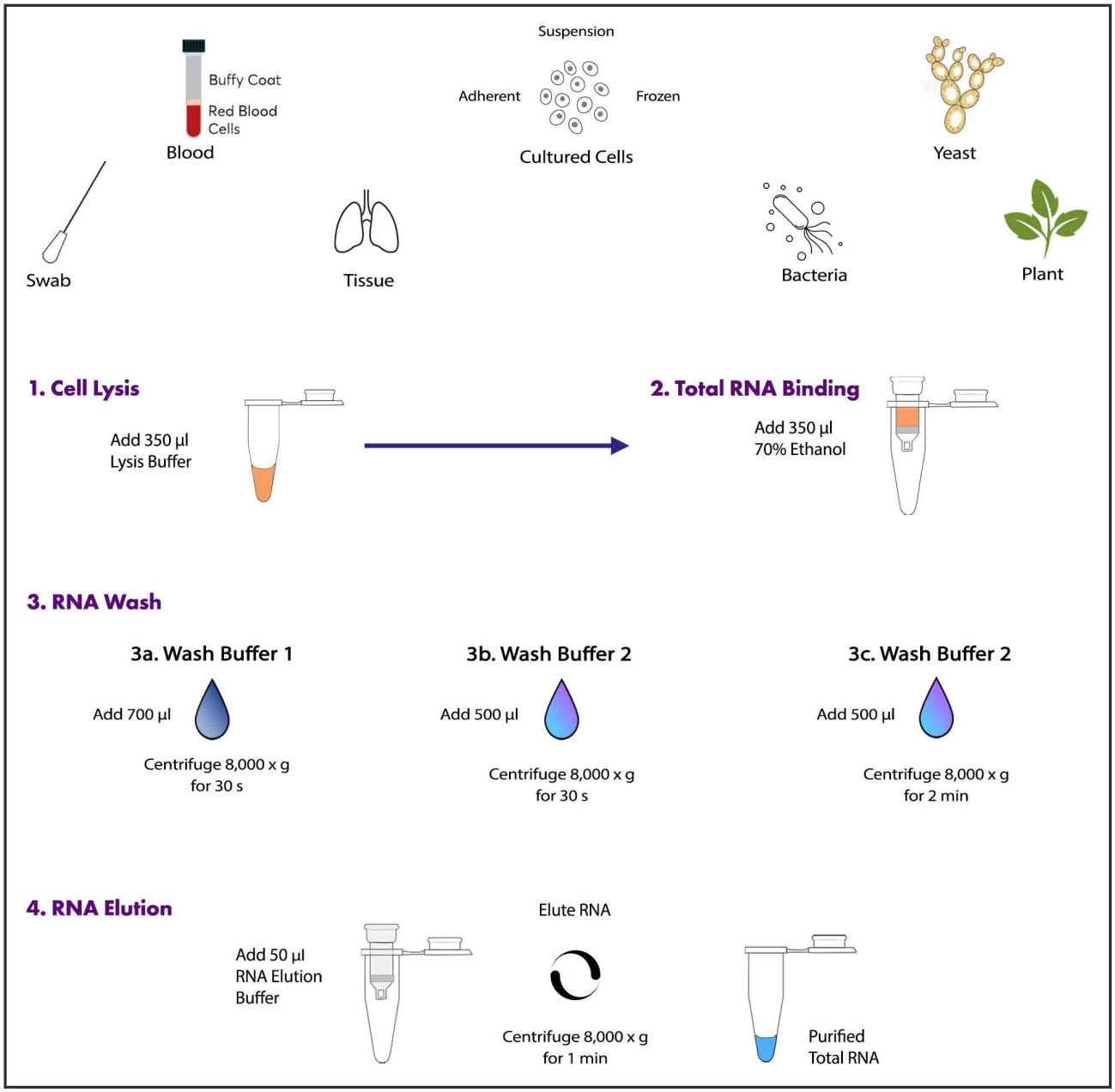



*Figure 1. Overview of RNA Extraction method*

# Kit Components

The Chromatrap® RNA Extraction kit contains reagents and spin columns to obtain pure RNA from up to 50 (Cat # 500335-050) or 250 (Cat # 500335-250) samples. The components of the kits are stable for up to 1 year when stored at the indicated temperature.

| Kit Component | Quantity | | Storage Temperature |
|---|---|---|---|
| | 50 Preps (500335-050) | 250 Preps (500335-250) | |
| RNA Extraction Lysis Buffer* | 30 ml | 150 ml | RT |
| RNA Extraction Wash Buffer 1 | 10 ml | 50 ml | RT |
| RNA Extraction Wash Buffer 2 | 20 ml | 100 ml | RT |
| RNA Elution Buffer | 4 ml | 20 ml | RT |
| RNA Extraction Columns | 50 | 250 | RT |
| Collection Tubes | 50 | 250 | RT |

*Table 1. RNA Extraction Kit Components*

**Lysis Buffer may need to be warmed to 37°C if precipitates have formed.*

## Caution

RNA Extraction Lysis Buffer & RNA Extraction Wash Buffer 1 contain guanidine salts and must be handled and disposed appropriately. These buffer are not compatible with bleach, please see MSDS for full details.

## Preparation of Buffers

Prepare buffers before first use. Tick box on label and include date when added.

*For Catalogue no. 500335-050*

1. Add **40 ml ethanol** (95-100%) to **10 ml RNA Extraction Wash Buffer 1** (total volume 50 ml)
2. Add **80 ml ethanol** (95-100%) to **20 ml RNA Extraction Wash Buffer 2** (total volume 100 ml)

*For Catalogue no. 500335-250*

1. Add **200 ml ethanol** (95-100%) to **50 ml RNA Extraction Wash Buffer 1** (total volume 250 ml)
2. Add **400 ml ethanol** (95-100%) to **100 ml RNA Extraction Wash Buffer 2** (total volume 500 ml)


*The Chromatrap® RNA Extraction kit is for research purposes only. Not intended for diagnostic use.*

# Additional Materials

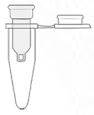
It is essential that RNase free equipment and consumables are used at all stages of the protocol. Any disruption equipment such as scalpel blades or mortar and pestles must be RNase free before use. The use of barrier tips is recommended.

Additional materials to be provided by the user

- Ethanol Absolute (95-100%)
- 70% Ethanol
- Microcentrifuge (4°C)
- RNase-free DNase & suitable digestion buffer (optional)
- ß-mercaptoethanol (ß-me) or 1M dithiothreitol (DTT) (optional)
- Chromatrap® Homogeniser Spin Column Cat # 500289 (optional)
- Chromatrap® gDNA Removal Column Cat # 500338 (optional)

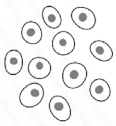
For cell disruption and sample homogenisation one or more of the following is required;

**N.B** It is essential to completely disrupt cell membranes and homogenise tissue samples to ensure efficient lysis and prevent blocking of the RNA capture membrane. The method of disruption depends on the sample type. General guidelines are given in the relevant section but this may need to be optimised by the user.



- Chromatrap® Homogeniser Spin Column (Cat # 500289)
- Needle and syringe
- Mortar and pestle
- Rotor-stator homogeniser
- Bead mill homogeniser

For cell lines;



- Trypsin (for adherent cell lines only)
- Cell scrapers (optional)
- PBS

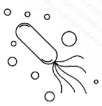
For plants;

- Pestle & mortar
- Liquid nitrogen

For yeasts;

- Zymolase or lyticase and suitable digestion buffer for enzymatic lysis
- Mortar and pestle
- Dry ice

For bacteria;



- 10 % SDS
- Lysozyme and suitable digestion buffer

# Protocol

Before Starting

***Clean working areas and pipettes thoroughly with suitable RNase degrading cleaning solution before starting the protocol. Use fresh gloves and change regularly. It is essential that RNase free equipment and consumables are used at all stages of the protocol. Any disruption equipment such as scalpel blades or mortar and pestles must be RNase free before use. The use of barrier tips is recommended. It is important to work as quickly and carefully as possible when handling material for RNA extraction to minimise degradation and maximise yield.***

## Section 1 | Sample Collection and Cell Lysis

For RNA extraction from tissue or cell lines considered to have high levels of RNases it is recommended to add ß-me to a final concentration of 0.01% (v/v) or DTT to a final concentration of 40 mM to RNA Extraction Lysis Buffer prior to addition to cell or tissue pellets.

The volume of RNA Extraction Lysis Buffer and appropriate disruption and homogenisation methods recommended are indicated by sample size and type in in Tables 2-7 in the following section.

### 1.1 Sample Collection & Lysis for Cultured Cells

For >$10^6$ cells or soft tissue we recommend increasing the volume of RNA Extraction Lysis buffer and 70% ethanol to 700 µl. Homogenising the sample using a Chromatrap® Homogeniser Spin Column (Cat # 500289) will reduce sample viscosity and increase RNA yield for larger sample sizes, see Appendix 1 for protocol.

| Sample | Cell Number | Lysis Buffer Volume | Sample Prep Method |
|---|---|---|---|
| Cultured Cells | <$10^6$ | 350 µl | Vortex |
| | <$10^6$ <$10^7$ | 700 µl | Spin Column Homogeniser |

*Table 2. Recommended RNA Extraction Lysis Buffer volume by cell number*

#### 1.1.1 Sample Collection & Lysis for Adherent Cells

1. Culture cells for 24-72 hours using appropriate culture conditions for cell line being used and desired experimental conditions.
2. Aspirate media from flask or plate using a pipette and discard media.
3. Wash cells once with warm PBS to remove residual media.
4. Add appropriate volume of trypsin to cover the cells and incubate 5 min at 37 °C.
5. Remove cells from the surface of the culture vessel by gently tapping or by scraping with a cell scraper.
6. Add appropriate volume of full media (3 volumes full media to1 volume trypsin) to the culture vessel to inactivate the trypsin.
7. Collect cell suspension by aspiration using a pipette and place in appropriate RNase free centrifuge tube.
8. Centrifuge cell suspension 2,000 x g, 5 min at 4°C to pellet cells. Discard supernatant.
9. Add correct volume of RNA Extraction Lysis Buffer and mix thoroughly by vortexing (<$10^6$ cells) or spin column homogenisation (>$10^6$ cells). Place sample on ice and proceed to Section 2.

### 1.1.2 Sample Collection & Lysis for Suspension Cells

1. Culture cells for 24-72 hours using appropriate culture conditions for cell line being used and desired experimental conditions.
2. Aspirate or decant cells in culture media, transfer to an appropriate tube and centrifuge 1,200 x g for 5 min at 4°C to pellet cells. Discard media.
3. Add correct volume of RNA Extraction Lysis Buffer and mix thoroughly by vortexing (<$10^6$ cells) or spin column homogenisation (>$10^6$ cells). Place sample on ice and proceed to Section 2.

### 1.1.3 Sample Collection & Lysis for Frozen Cell Pellets

1. Place tube containing frozen cell pellets on ice and allow to thaw very slightly. The pellet should be easily dislodged by agitation of the tube but not completely thawed to protect the integrity of the RNA.
2. Add correct volume of RNA Extraction Lysis Buffer and mix thoroughly by vortexing (<$10^6$ cells) or spin column homogenisation (>$10^6$ cells). Ensure sample is completely lysed and no cell debris remains.
3. Place sample on ice and proceed to Section 2.

## 1.2 Sample Collection & Lysis for Animal Tissue

For preparation of RNA from tissue samples it is recommended to add ß-me to a final concentration of 0.1 % (v/v) or DTT to a final concentration of 40 mM to RNA Extraction Lysis Buffer when lysing tissue.

**N.B.** Frozen tissue should be kept frozen during extraction to maintain RNA integrity. Sample tubes should be placed on ice during homogenisation, ideally on dry ice.

| Sample | Tissue Size | Lysis Buffer Volume | Sample Prep Method |
|---|---|---|---|
| Soft Tissue (Fresh) | <25 mg | 350 µl | Bead mill or Rotor-stator followed by Spin Column Homogeniser |
| | >25 mg <50 mg | 700 µl | |
| | >50 mg <100 mg | 350 µl/25 mg Tissue | Rotor-stator followed by Spin Column Homogeniser |
| Soft Tissue (Frozen) | < 10 mg | 350 µl | Bead mill or Rotor-stator followed by Spin Column Homogeniser |
| | > 10 mg <50 mg | 700 µl | Rotor-stator followed by Spin Column Homogeniser |
| Fibrous Tissue (Fresh or Frozen) | < 10 mg | 350 µl | Rotor-stator followed by Spin Column Homogeniser |
| | > 10 mg <50 mg | 700 µl | |

*Table 3. Recommended RNA Extraction Lysis Buffer volume by cell number*

### 1.2.1 Sample Collection & Lysis for Fresh or Frozen Soft Tissue

1. Pre-cool an appropriate volume centrifuge tube on ice. Place tissue sample in the tube and add correct volume of RNA Extraction Lysis Buffer for the weight of tissue.
2. Disrupt tissue sample using method (2a), (2b) or (2c);
   (2a) Disrupt tissue suspension for 30-40 s with rotor stator homogeniser
   (2b) Pass the lysate through a syringe needle 5-10 times
   (2c) Load tissue lysate into a bead mill tube and disrupt tissue using a suitable bead beating instrument or vortex for 10 min.
3. Homogenise sample using a spin column homogeniser (OPTIONAL: homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).
4. Place sample on ice and proceed to Section 2.

### 1.2.2 Sample Collection & Lysis for Fresh or Frozen Fibrous Tissue

1. Pre-cool an appropriate volume centrifuge tube on ice. Place tissue sample in the tube and add correct volume of RNA Extraction Lysis Buffer for the weight of tissue.
2. Disrupt tissue sample using method (2a) or (2b).
   (2a) Disrupt tissue suspension for 30-40 s with rotor stator homogeniser
   (2b) Pass the lysate through a syringe needle 5-10 times
3. Homogenise sample using a spin column homogeniser (OPTIONAL: homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).
4. Place sample on ice and proceed to Section 2.

## 1.3 Sample Collection & Lysis for Whole Blood

It is recommended to collect blood in the presence of anticoagulants such as EDTA, citrate or heparin. To maximise yield and minimise degradation where possible samples should be processed for RNA extraction within a few hours of collection. Frozen whole blood samples can be used for RNA extraction but it is important to note the yield and quality from frozen blood samples may be lower.

**N.B. 10 µl ß-Me** per ml of RNA Extraction Lysis Buffer must be added prior to use when working with whole blood.

| Sample | Sample Volume | Lysis Buffer Volume | Sample Prep Method |
|---|---|---|---|
| Fresh or Frozen Whole Blood | ≤0.25 ml | 350 µl | Spin Column Homogeniser |
| | ≤0.25 ml <0.5 ml | 700 µl | Spin Column Homogeniser |

*Table 4. Recommended RNA Extraction Lysis Buffer volume by blood volume*

1. Transfer desired volume of whole blood to a 1.5 ml microcentrifuge tube.
2. Add correct volume of RNA Extraction Lysis Buffer and mix thoroughly by pipetting or vortexing, taking care to ensure no aggregates have formed and dispersing any visible clumps.

   **N.B** Ensure **10 µl ß-me** has been added per ml of **RNA Extraction Lysis Buffer** before use.
3. Homogenise sample using a spin column homogeniser (OPTIONAL: homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).
4. Place sample on ice and proceed to Section 2.

## 1.4 Sample Collection & Lysis for Buccal Swabs

For preparation of RNA from buccal swabs it is recommended to add ß-me to a final concentration of 0.1 % (v/v) or DTT to a final concentration of 40 mM to RNA Extraction Lysis Buffer.

1. Cut the buccal swab into a 1.5 ml microcentrifuge tube. Add 400 µl RNA Extraction Lysis Buffer and vortex for 1 min.
2. Transfer the lysate and swab to a Chromatrap® Homogeniser Spin Column (in a 2 ml collection tube). Centrifuge for 5 min at maximum speed. Note: It is important to transfer the swab and the lysate on to the homogeniser spin column, to avoid any loss of RNA.
3. Proceed to Section 2 using the lysate that has flowed through the Chromatrap® Homogeniser Spin Column and is contained in the 2 ml collection tube.

## 1.5 Sample Collection & Lysis for Plants

It is recommended to add **10 µl ß-Me** per ml of RNA Extraction Lysis Buffer when working with plant material. The recommended volumes of RNA Lysis Extraction for weight of tissue are given in Table 5 below. When working with frozen tissue is essential the tissue is not allowed to thaw prior to lysis and disruption. If disrupting tissue with pestle and mortar then flash freezing tissue with liquid nitrogen before disruption is recommended. It is important to work quickly whilst disrupting tissue to prevent loss and degradation of RNA.

| Sample | Tissue Size | Lysis Buffer Volume | Sample Prep Method |
|---|---|---|---|
| Soft tissue (Fresh) | ≤100 mg | 0.5 ml | Scalpel blade and rotor-stator followed by Spin Column Homogeniser |
| | >100 mg <200 mg | 1.0 ml | |
| | < 250 mg | 1.5 ml | |
| Soft tissue (Frozen) and Fibrous tissue (Fresh or Frozen) | ≤100 mg | 0.5 ml | Mortar and pestle followed by Spin Column Homogeniser |
| | >100 mg <200 mg | 1.0 ml | |
| | <250 mg | 1.5 ml | |

*Table 5. Recommended RNA Extraction Lysis Buffer volume by plant tissue weight and type*

## 1.5.1 Sample Collection and Lysis for Fresh Soft Plant Tissue

1. Carefully slice tissue into very small pieces on ice. Transfer tissue fragments to an appropriate volume pre-cooled centrifuge tube and add correct volume of RNA Extraction Lysis Buffer for the weight of tissue.
2. Disrupt tissue sample 45-60 s with rotor stator homogeniser.
3. Process disrupted tissue using method (3a) or (3b).

   (3a) Homogenise sample using a Spin Column Homogeniser (homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).

   (3b) Centrifuge the disrupted sample 5 min, 3,000 x g at room temperature to pellet cell debris. Transfer only the clear supernatant to a new 1.5 ml microcentrifuge tube taking care not to disturb the pellet.
4. Place sample on ice and proceed to Section 2.

## 1.5.2 Sample Collection & Lysis for Fresh Fibrous Plant Tissue

5. Carefully slice tissue into very small pieces on ice. Transfer tissue fragments to a mortar and add liquid nitrogen.
6. Grind the frozen tissue into a fine power using a pestle and transfer the powdered tissue to a new microcentrifuge tube.
7. Allow residual liquid nitrogen to evaporate then immediately add appropriate volume of RNA Extraction Lysis Buffer.

   **N.B** Ensure **10 µl ß-me** has been added per ml of **RNA Extraction Lysis Buffer** before use.
8. Process disrupted tissue using method (4a) or (4b);

   (4a) Mix sample thoroughly by pipetting or vortexing and homogenise using a Spin Column Homogeniser (homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).

   (4b) Homogenise tissue sample 45-60 s with rotor stator homogeniser and centrifuge 5 min, 3,000 x g at room temperature to pellet cell debris. Transfer only the clear supernatant to a new 1.5 ml microcentrifuge tube taking care not to disturb the pellet.
9. Place sample on ice and proceed to Section 2.

## 1.5.3 Sample Collection and Lysis for Frozen, Soft or Fibrous Plant Tissue

1. Taking care not to allow the frozen tissue to thaw transfer it to a mortar and add liquid nitrogen.
2. Grind the frozen tissue into a fine power using a pestle and transfer the powdered tissue to a new microcentrifuge tube.
3. Allow residual liquid nitrogen to evaporate then immediately add appropriate volume of RNA Extraction Lysis Buffer.

   **N.B** Ensure **10 µl ß-me** has been added per ml of **RNA Extraction Lysis Buffer** before use.
4. Process disrupted tissue using method (4a) or (4b);

   (4a) Mix sample thoroughly by pipetting or vortexing and homogenise using a Spin Column Homogeniser (homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).

   (4b) Homogenise tissue sample 45-60 s with rotor stator homogeniser and centrifuge 5 min, 3,000 x g at room temperature to pellet cell debris. Transfer only the clear supernatant to a new 1.5 ml microcentrifuge tube taking care not to disturb the pellet.
5. Place sample on ice and proceed to Section 2.

## 1.6 Sample Collection & Lysis for Yeasts

It is recommended to add **10 µl ß-Me** per ml of **RNA Extraction Lysis Buffer** when working with yeast cells. The recommended volumes of RNA Lysis Extraction for yeast cell numbers and the options for cell disruption are in Table 6 below. Yeast cells can be lysed by either enzymatic or mechanical methods, both methods produce comparable results however, it is not recommended to use enzymatic lysis for kinetic experiments. It is important to work quickly whilst disrupting yeast cells to prevent loss and degradation of RNA.

| Sample | Cell Number | Lysis Buffer Volume | Lysis Method | Sample Prep Method |
|---|---|---|---|---|
| Yeasts | ≤5 x $10^7$ | 500 µl | Enzymatic | - |
| | ≤5 x $10^7$ | 700 µl | Mechanical | Bead mill or mortar and pestle and Spin Column Homogeniser |

*Table 6. Recommended RNA Extraction Lysis Buffer volume by yeasts weight and type*

### 1.6.1 Sample Collection And Enzymatic Lysis For Yeasts

1. Grow yeast cells in desired conditions, yeast cells should be in log phase for harvesting.
2. Prepare digestion buffer for the zymolase, lyticase or other suitable enzyme to be used according to the manufacturers instructions. Add an appropriate amount of enzyme to the buffer for your number of cells following the enzyme manufacturers recommendations.
3. Harvest a maximum of 5 x $10^7$ yeast cells by centrifugation 800 x g, 5 min at 4°C.
4. Decant and discard the supernatant. Carefully aspirate any remaining liquid from above the pellet without dislodging it. Complete removal of residual media is essential for an efficient lysis reaction.
5. Resuspend the cell pellet in 250 µl digestion buffer (ensure **digestion enzyme** has been added to **digestion buffer** before use) and incubate 30 – 60 min at 30 °C. The length of incubation will depend on the enzyme and the type of cells being lysed, refer to the enzyme manufacturers instructions for guidance.
6. Add 500 µl RNA Extraction Lysis Buffer to the cell lysate and mix thoroughly by vortexing.
7. Pellet any cell debris by centrifuging at full speed for 2 min at room temperature. Transfer the **cleared supernatant** only to a fresh microcentrifuge tube and proceed to Section 2.

### 1.6.2 Sample Collection And Mechanical Lysis For Yeasts

1. Grow yeast cells in desired conditions, yeast cells should be in log phase for harvesting.
2. Harvest a maximum of 5 x $10^7$ yeast cells by centrifugation 800 x g, 5 min at 4°C. Decant and discard the supernatant. Carefully aspirate any remaining liquid from above the pellet without dislodging it. Complete removal of residual media is essential for an efficient lysis reaction.
3. Add 700 µl RNA Extraction Lysis Buffer to the pellet and resuspend the lysate thoroughly.
4. Process cell lysate using method (4a) or (4b);

   (4a) Crush a small amount of dry ice to a fine powder (approx. 10 g) in a mortar using a pestle. Add the lysate gradually (approx. 50-100 µl at a time) to the mortar grinding the droplets as they freeze. Crush the frozen lysate using the pestle until a fine power is formed then allow to dry ice to evaporate. Transfer the resulting paste to a spin column homogeniser (homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).

(4b) Add the entire lysate (~700 µl) to a bead mill and homogenise using a vortex or suitable bead beating instrument. It is essential to keep the sample cooled during this process. Allow the beads to settle and transfer cleared supernatant (avoiding the beads) to a spin column homogeniser (homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).

5. Transfer the cleared supernatant only to a fresh microcentrifuge tube and proceed to Section 2.

## 1.7 Sample Collection & Lysis for Bacteria

It is recommended to add **10 µl ß-Me** per ml of RNA Extraction Lysis Buffer when working with bacterial cells. The recommended volumes of RNA Lysis Extraction for up to 5 x $10^7$ bacterial cells and the disruption and homogenisation methods are shown in Table 7 below. It is important to work quickly whilst disrupting bacterial cells to prevent loss and degradation of RNA.

Before starting the extraction prepare sufficient lysozyme solution (10 mg/ml lysozyme in 10 mM Tris HCl pH8) solution for the number of samples being processed. 100 µl lysozyme solution is required per sample.

| Sample | Cell number | Lysis Buffer Volume | Sample Prep Method |
|---|---|---|---|
| Bacteria | ≤5 x $10^8$ | 350 µl | Lysozyme digestion followed by Spin Column Homogeniser, syringe and needle or rotor stator |

*Table 7. Recommended RNA Extraction Lysis Buffer volume by bacterial cell number*

1. Grow bacterial cells in desired conditions, bacterial cells should be in log phase for harvesting.
2. Harvest a maximum of 5 x $10^8$ bacterial cells by centrifugation 800 x g, 5 min at 4 °C. Decant and discard the supernatant. Carefully aspirate any remaining liquid from above the pellet without dislodging it. Complete removal of residual media is essential for an efficient lysis reaction.
3. Resuspend the cell pellet in 100 µl lysozyme solution and vortex to mix thoroughly.
4. Add 0.5 µl 10 % SDS, mix thoroughly by vortexing and incubate the cell suspension 5 min at room temperature.
5. Add 350 µl RNA Extraction Lysis Buffer to the cell suspension and mix thoroughly by vortexing.
6. Disrupt cells using method (6a), (6b) or (6c).

   (6a) Transfer cell lysate to a spin column homogeniser (homogenisation of disrupted sample using a Chromatrap® Spin Column Homogeniser can significantly improve yield, see Appendix 1 for protocol).

   (6b) Pass the cell lysate through a syringe needle 5-10 times or

   (6c) Disrupt cell lysate for 30-40 s with rotor stator homogeniser.
7. Transfer the **cleared supernatant** only to a fresh microcentrifuge tube and proceed to Section 2.

# Section 2 | Extraction and Purification of Total RNA

All steps of Section 2 should be performed quickly but carefully at room temperature to maximise RNA yield and minimise sample degradation.

## RNA Capture, Washing and Elution

1. Place an RNA Extraction spin column in a 2 ml collection tube.
2. (OPTIONAL) Load a maximum of 700 µl sample onto a gDNA removal column (Chromatrap® Cat # 500338) and centrifuge 12,000 x g for 30 s. Aspirate flowthrough (contains RNA) and transfer to a fresh microcentrifuge tube.
3. Follow step (3a) or (3b) depending on the sample source to prepare the Lysis Buffer suspension for binding;(3a) **For animal tissue, whole blood, yeasts, bacteria or cell lines add 1 volume** (eg. 350 µl for cell suspensions in 350 µl RNA Extraction Lysis Buffer) of 70 % ethanol to the RNA Extraction Lysis Buffer cell suspension and mix thoroughly by pipetting.

   (3b) **For plant tissue add 0.5 volumes 96-100 % ethanol** to the RNA Extraction Lysis Buffer cell suspension and mix thoroughly by pipetting.

**N.B.** Precipitates may form at this stage but will not affect the efficiency of RNA binding. Load any precipitates onto the RNA Extraction column along with the lysate.

4. Load a maximum of 700 µl sample immediately onto spin column. (If using larger volumes load ≤700 µl, spin through and discard the flow through, load the remainder of the sample onto the same RNA Extraction column).
5. Centrifuge 10,000 x g 30 s. Discard flowthrough.
6. Add 700µl RNA Extraction Wash Buffer 1, centrifuge 10,000 x g 30 s and discard flowthrough.
7. (OPTIONAL) Perform on-column DNase digestion according to section C overleaf.
8. Add 500 µl RNA Extraction Wash Buffer 2, centrifuge 10,000 x g 30 s and discard flowthrough.
9. Add 500 µl RNA Extraction Wash Buffer 2, centrifuge 10,000 x g 2 min and discard flowthrough.
10. (OPTIONAL) Perform a dry spin, 1 min full speed.
11. Transfer spin column to a clean dry 1.5 ml microcentrifuge tube. Add 50 µl RNA Elution Buffer directly to the membrane.
12. Elute RNA by centrifuging 1 min 8,000 x g.

Purified RNA is now ready for downstream applications or can be stored at -80 °C for up to 1 year.

# Additional Protocols

## RNA Clean Up

The following protocol can be used to further purify RNA which has already been extracted or from reaction mixtures such as DNAse digestion.

1. Add 350 µl RNA Extraction Lysis Buffer to ≤100 µg extracted RNA or reaction mixture.
2. Place an RNA Extraction spin column in a 2 ml collection tube.
3. Add 350 µl 70% ethanol to sample in RNA Extraction Lysis Buffer, mix well and load entire 700 µl sample immediately onto spin column.
4. Centrifuge 8,000 x g 30 s. Discard flowthrough.
5. Add 700 µl RNA Extraction Wash Buffer 1, centrifuge 8,000 x g for 30 s and discard flowthrough.
6. Add 500 µl RNA Extraction Wash Buffer 2, centrifuge 8,000 x g for 30 s and discard flowthrough.
7. Add 500 µl RNA Extraction Wash Buffer 2, centrifuge 8,000 x g for 2 min and discard flowthrough.
8. (OPTIONAL) Perform a dry spin, 1 min full speed.
9. Transfer spin column to a clean dry 1.5 ml microcentrifuge tube. Add 50 µl RNA Elution Buffer water directly to the membrane.
10. Elute RNA by centrifuging 1 min 8,000 x g.

RNA is now ready for downstream processing. Store RNA at -80 °C if not using immediately.

## DNase Digestion

1. For DNA-sensitive applications an on-column DNase 1 digestion step can be performed as follows;
2. Add 50 U DNase 1 to100 µl suitable DNase 1 digestion buffer* and mix well.
3. Load directly onto the membrane of the RNA Extraction column and incubate 15 min at room temperature.
4. Add 700 µl RNA Wash Buffer 1 and centrifuge 1 min 10,000 x g.
5. Continue protocol from Step 8 of Section 2 | RNA Extraction (page 14).

*Example DNase 1 digestion buffer; 10 mM Tris-HCl, 5 mM EDTA, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6.

# Troubleshooting and FAQs

### Can I elute in less than 50 µl RNA Elution Buffer?

The volume of elution buffer can be reduced to a minimum of 10 µl to obtain a more concentrated RNA sample, this may result in reduced total yield of RNA.

### Can I elute in more than 50 µl RNA Elution Buffer?

Is it possible to elute in greater volumes than 50 µl although it is important to note this may result in lower concentration RNA samples. For recovery of RNA from large amounts of starting material expected to yield >100 µg RNA it is recommended to elute in 100 µl RNA Elution Buffer. For maximum yield (up to 1000 µg RNA) it is recommended to perform sequential elutions of 100 µl using the same RNA Extraction column up to a maximum of 3 times (3 x 100 µl elutions). When performing sequential elutions collect all eluates in the same collection tube.

### Why is my sample very viscous after addition of RNA Extraction Lysis Buffer and/or contains lumps of debris?

Ensure sample is mixed thoroughly and cells are fully resuspended in RNA Extraction Lysis Buffer. If debris is visible in the sample or the sample is very viscous inadequate Lysis Buffer for the number of cells may have been used. Increase volume of RNA Extraction Lysis Buffer to 700 µl for each sample. Complete lysis of cells and suspension of cell debris is critical for yield and purity of extracted RNA. For high numbers of cells or viscous samples homogenisation of the sample using a Chromatrap® Homogeniser Spin Column (Cat # 500289) is recommended to maximise yield and purity.

### Why do I have poor yield of RNA?

Ensure sample is fully lysed and appropriate volume of RNA Extraction Buffer has been used. Check ethanol has been added to both wash buffers if this is the first use. Consider using a Chromatrap® Homogeniser Spin Column (Cat # 500289) for high input samples or increase input material for low input samples.

### Why is the purity of my RNA sample high/low?

Sample was not fully lysed and/or cell debris was loaded onto the column, see **'Why do I have poor yield of RNA?'** above.

Salt carryover may occur due to incorrect sample handling. Use fresh gloves from addition of RNA Extraction Wash Buffer 2 wash one and do not touch the inside or bottom of the spin column containing the membrane. Ensure the inside of the spin column is wash thoroughly when loading the wash buffers.

Ethanol carryover can be eliminated by performing the optional dry spin (Point 10, Section 2, page 14).

Note: Pure RNA should have an $A_{260/280}$ of ~ 2 and $A_{260/230}$ of ~2-2.2. Partial lysis and carryover of guanidine salts or ethanol are the most common causes of poor purity RNA preparations.

### Why is my RNA degraded?

RNases are ubiquitous in the environment, especially on hands. Clean works areas and pipettes with suitable RNase degrading cleaning solution. Use fresh gloves and change regularly. Use only microcentrifuge tubes and pipette tips which are certified RNase free. The use of barrier tips is highly recommended. Work quickly and maintain samples on ice where possible to protect the integrity of the RNA. All reagents, columns and tubes in the Chromatrap® RNA Extraction kit are certified RNase-Free. Use only the supplied RNA Elution Buffer or RNase free water to elute the RNA.

### What temperature should I store my RNA?

RNA should be stored at -80 °C to prevent degradation.

### Why did the column become blocked during centrifugation after sample loading and what can I do?

Too much sample material was used with insufficient Lysis Buffer. Increase the volume of RNA Extraction Lysis Buffer added to the cell or tissue sample. Ensure sample is thoroughly lysed with no visible debris before loading onto the column. If using >$10^6$ cells or soft tissue the use of Chromatrap® Spin Column Homogenisers (Cat # 500289) is recommended to aid Lysis and reduce sample viscosity.

It is essential that samples are handled and all centrifugation steps are carried out at room temperature during Section 2| Extraction of Total RNA. Lower temperatures can cause precipitates to form in the RNA Extraction Lysis buffer which can cause the column to block during sample binding.

### I have a large number of samples to test, is there a high throughput version of the kit I could use?

The Chromatrap® RNA Extraction kit is available in 96 well plate format, Chromatrap® RNA 96 Extraction kit Catalogue # 500335-096, for rapid isolation of RNA from up to 96 samples in parallel.

# Appendix 1

## Chromatrap® Homogeniser Spin Column Protocol

1. Position the Chromatrap® Homogeniser Spin Column in the collection tube provided.
2. Transfer up to 700 µl cell or tissue lysate (cells or disrupted tissue in RNA Extraction Lysis Buffer) to the Chromatrap® Homogeniser Spin Column and close the cap.
3. Centrifuge at full speed for 2 min at room temperature.
4. Remove and discard the Homogeniser Spin Column from the collection tube. Do not discard the flowthrough, this contains the homogenised lysate.
5. Carefully aspirate the suspension from the collection tube using a pipette, avoiding any cell debris, and transfer to a fresh clean 1.5 ml microcentrifuge tube.
6. Sample is now ready for downstream processing, place sample on ice and continue protocol from Section 2 (page 18) Extraction of Total RNA:RNA capture, washing and elution.

**N.B.** if using >700 µl RNA Extraction Lysis Buffer use a new Chromatrap® Homogeniser Spin Column for each 700 µl of lysate, performing steps 1-5 with each column. Pool the flowthrough from each Chromatrap® Homogeniser Spin Column into the same microcentrifuge tube and process as one sample for RNA extraction.

# Related Chromatrap® Products

| Product | Quantity | Catalogue No. |
|---|---|---|
| Chromatrap® Spin Column Homogeniser | 50 | 500289 |
| Chromatrap® gDNA Removal Column | 50 | 500338 |

# Other Chromatrap® Products

## DNA Products

| Product | Quantity | Catalogue No. |
|---|---|---|
| Chromatrap® DNA Purification | 50 | 500189 |
| Chromatrap® Gel Purification | 50 | 500190 |
| Chromatrap® HT DNA Purification | 2 x 96 | 500214 |
| Chromatrap® HT DNA Purify and Concentrate | 2 x 96 | 500215 |
| Chromatrap® DNA Extraction | 50 | 500191 |
| Chromatrap® HT DNA Extraction | 2 x 96 | 500192 |
| Chromatrap® Size Selection | 50 | 500216 |
| Chromatrap® HT Size Selection | 2 x 96 | 500217 |

## ChIP Products

| Product | Quantity | Catalogue No. |
|---|---|---|
| Chromatrap® ChIP-Seq Pro A | 24 | 500189 |
| Chromatrap® ChIP-Seq Pro G | 24 | 500190 |
| Chromatrap® HT ChIP-Seq Pro A | 1 x 96 | 500214 |
| Chromatrap® HT ChIP-Seq Pro G | 1 x 96 | 500215 |
| Chromatrap® Enzymatic ChIP-seq Pro A | 24 | 500191 |
| Chromatrap® Enzymatic ChIP-seq Pro G | 24 | 500192 |
| Chromatrap® HT Enzymatic ChIP-seq Pro A | 1 x 96 | 500216 |
| Chromatrap® HT Enzymatic ChIP-seq Pro G | 1 x 96 | 500217 |
| Chromatrap® FFPE ChIP-seq Pro A | 24 | 500235 |
| Chromatrap® FFPE ChIP-seq Pro G | 24 | 500236 |
| Chromatrap® Native ChIP-seq Pro A | 24 | 500237 |
| Chromatrap® Native ChIP-seq Pro G | 24 | 500238 |
| Chromatrap® UniqSeq Pro A | 24 | 500264 |
| Chromatrap® UniqSeq Pro G | 24 | 500265 |
| Chromatrap® UniqSeq Enzymatic Pro A | 24 | 500266 |
| Chromatrap® UniqSeq Enzymatic Pro G | 24 | 500267 |
| Chromatrap® *Drosophila* ChIP-Seq Pro A | 24 | 500279 |
| Chromatrap® *Drosophila* ChIP-Seq Pro G | 24 | 500275 |
| Chromatrap® *Drosophila* UniqSeq Pro A | 24 | 500276 |
| Chromatrap® *Drosophila* UniqSeq Pro G | 24 | 500277 |
| Chromatrap® Sonication Shearing | - | 500239 |
| Chromatrap® Enzymatic Shearing | - | 500165 |

Chromatrap

www.chromatrap.com

**Worldwide Chromatrap® Technical Support Team**
support@chromatrap.com

**Worldwide Sales and Customer Support Team**
sales@chromatrap.com
Tel: +44 (0) 1978 666222
Clywedog Road South Wrexham Industrial Estate Wrexham LL13 9XS UK

*Chromatrap is a registered trademark of Porvair plc. Vyon is a trademark of Porvair plc. Chromatrap® uses novel patented technology (UK Patent No. GB2482209, & GB2527623, US Patent No. 9523681 & 10,435,480, Chinese Patent No. ZL 2011 8 0067254.X, Japan Patent No. JP 6088434 and Australian Patent No. AU 2011340263). © Copyright 2021. Porvair Filtration Group Ltd. All rights reserved. Whilst every effort has been made to ensure the accuracy of this document, due to continuous product development, the data contained is subject to constant revision and Porvair Sciences Ltd. reserves the right to change, alter or modify its contents.*