GENETIX BRAND

HANDBOOK

Nucleo-pore™

Total RNA Purification from Plant

☐ **RNASure® Plant Kit**	NP-84905	50 Preps
☐ **RNASure® Plant Kit**	NP-84907	250 Preps

genetix Biotech Asia Pvt. Ltd.

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Nucleo-pore™

Table of Contents

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KIT CONTENTS

RNA Sure Plant

Cat. No.	NP-84905	NP-84907
Number of Preps	50	250
Wash Buffer RWB2	15 ml	80 ml
Lysis Buffer RLB1	25 ml	125 ml
Lysisi Buffer RLB-P	25 ml	125 ml
Wash Buffer RWB3 (conc)*	12.5 ml	3 x 25 ml
Desalting Buffer RDB	25 ml	125 ml
Reaction buffer for rDNase	7 ml	35 ml
rDNase,RNase-free (lyophilised)*	1 vial (size D)	1 vial (sizeD)
RNase-free Water	15 ml	65 ml
RNASure® Shredder	50	250
RNASure® Plant Column	50	250
Collection tubes(2ml)	150	750
Collection tube (1.5ml)	50	250
Handbook	1	1

* Please see “Preparation of Reagents”

Reagents, consumables, and equipment not supplied with the kit

- 96 - 100 % ethanol (to prepare Wash Buffer RWB3)
- 70 % ethanol (to adjust RNA binding conditions)
- ß-mercaptoethanol, or DTT (dithiothreithol),
- 1.5 ml microcentrifuge tubes
- Sterile RNase-free pipette tips
- Manual pipette
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (lab coat, gloves, goggles)

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs).

Buffers RLB1,RLB-P and RWB2 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. The following risk and safety phrases apply to components of the RNASure® Plant Kit:

Buffer RLB1
Contains Guanidium thiocyanate: R&S Phrases: R20/21/22, S13

Buffer RLB-P
Contains Guanidium hydrochloride: R&S Phrases: R22-36/38

Buffer RWB2
Contains Guanidium thiocyanate: R&S Phrases: R10-20/21/22, S7-13-16

Desalting Buffer RDB
Contains Guanidium thiocyanate <10% + ethanol <10%: R&S Phrases: R10, S7-16

rDNase
Contains lyophilized rDNase: R&S Phrases: R42/43, S22-24

R10: Flammable, R20/21/22: Harmful by inhalation, in contact with skin and if swallowed, R36/38: Irritating to eyes and skin, May cause sensitization by inhalation and skin contact, R42/43: May cause sensitization by inhalation and skin contact, S7: Keep container tightly closed, S13: Keep away from food, drink and animal foodstuffs, S16: Keep away from sources of ignition - No smoking, S22: Do not breathe dust, S24: Avoid contact with skin.

INTRODUCTION

Principle and Procedure

RNASure® Plant Mini Kit provide a fast and easy way to purify DNA from plant and fungal tissue. Up to 100 mg of tissue can be processed using the DNeasy Plant Mini Kit. Cells are disrupted by grinding in presence of liquid nitrogen. The lysed samples are then incubated with buffers containing chaotropic salts which are capable of protecting RNA from endogenous RNAses. Contamination of DNA is removed by on-column RNase free rDNase treatment. The samples are then processed through a spin cartridge containing clear silica based membrane to which RNA binds. Any impurities are removed by subsequent two washing steps. Pure RNA is then eluted with RNase-free water supplied with the kit, which can further be used in variety of downstream applications. The RNASure® Plant kit contain two different lysis buffers RLB1 and RLB-P respectively. For all general applications buffer RLB1 is recommended for Lysis. In certain plant tissue and fungi peculiar metabolites are present, then buffer RLB is optimized for lysis of samples containing peculiar metabolites. All RNA preparation steps can be performed at room temperature. The final eluate should be kept frozen at -20°C for short term storage and keep it at -70°C for long term storage to prevent RNA degradation.

Specifications of RNASure® Plant Kit

RNASure® Plant kit is simple reliable and rapid method for the isolation of total RNA from plant cells and tissues or filamentous fungi. Within 30 minute high quality of RNA can be prepared from 10mg of plant tissues. If used in RT-PCR applications we recommend using intron-spannig primers. The kit is supplied with rDNase for an on-column digestion to have minimal DNA contamination. An optional digestion with rDNase can be performed for most demanding applications.

Starting material and typical yield

Upto 70µg of RNA can be extracted from 100mg of starting material with an elution volume of 60µl using RNASure ® Plant Mini Kit.

Homogenization of Plant Samples

Plant Tissues

Complete disruption of cell walls and plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Depending upon the sample type disruption can be done mechanically. For disruption using a mortar and pestle, freeze the animal tissue immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into a liquid-nitrogen–cooled, appropriately sized tube and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add lysis buffer RLB1 containing beta mercaptoethanol and mix immediately. Then pass lysate through RNASure Shredder (included in the kit) or through 0.9mm syringe needle. At least 5–10 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may be required to facilitate handling and minimize loss. Rotor–stator homogenizers disrupt and homogenize simultaneously in the presence of lysis buffer RLB1, single samples of animal tissues in 15–90 seconds depending on the toughness and size of the sample. Rotor–stator homogenizers can also be used to homogenize cell lysates. The rotor turns at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. Foaming of the sample should be kept to a minimum by using properly sized vessels, keeping the tip of the homogenizer submerged, and holding the immersed tip to the side of the tube. Rotor–stator homogenizers are available in different sizes and operate with differently sized probes. Probes with diameters of 5 mm and 7 mm are suitable for volumes up to 300 µl and can be used for homogenization in microcentrifuge tubes. Probes with a diameter of 10 mm or above require larger tubes. In addition, round-bottomed tubes allow more efficient homogenization than conical bottomed.
Maintain RNase free environment during the entire process.

Preparation of Reagents

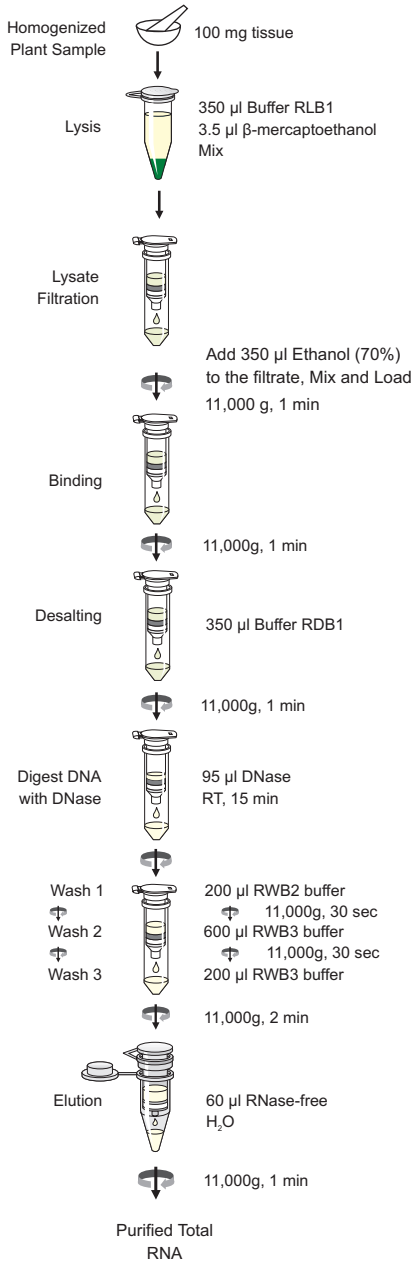
Always wear gloves & safety goggles as buffers contain guanidine salts.

- All buffers & kit components are stable at room temperature (18°C-25°C) for at least 12 months, Lyophilized rDNase (RNase free) at 4°C on arrival (it is stable upto 12 months).
- Keep up 70% ethanol ready for use in protocol.

Reconstitute Wash Buffer RWB3
Reconstitute Buffer RWB3 concentrate by adding, 50ml (Kit NP-84905) and 100ml to each bottle (Kit NP-84907) ethanol (96-100%). Mark the bottle as “Ethanol Added”. Buffer RWB3 can be stored at room temperature (18-25°C) for at least one year.

rDNase
Add 540ul of RNase Freewater to each rDNase vial and incubate for one minute at room temperature (18-25°C). gently mix to dissolve the rDNase completely. Dispense into aliquots and store at -20°C. the frozen sample is stable for 6 months. Do not freeze thaw more than three times.

RNA Purification from Plant



Protocol for Total RNA isolation from plant tissue or Filamentous fungi

Total RNA purification with RNASure® Plant Kit

Before starting the preparation:

- Check if Wash Buffer RWB3 and rDNase were prepared as per instructions

Procedure

1. Add liquid nitrogen to RNase free mortar and grind well upto 100mg tissues. Homogenize upto 100mg of plant material in a precooled mortar and pestle and grind well.

2. Add 350µl buffer RLB1 and 3.5µl ß-mercaptoethanol to the homogenized 100mg of tissues from step 1 and vortex well.
   Note: As alternative to ß-ME the reducing agent DTT or TCEPmay be used. Use a final concentration of 10 - 20 mM DTT or TCEP within the Lysis Buffer RLB1 or RLB.

3. Place the RNASure® Shredder in collection tube (2ml), apply the mixture and centrifuge at 11,000 x g for 1 min. Transfer the filtrate to a new centrifuge tube 1.5 ml capacity (not provided but available from genetix brand).
   Note : Do not disturb the pellet of cell debris, which may be visible after centrifugation. Transfer the pellet to a new 1.5ml centrifuge tube (not provided but available from genetix brand).

   Discard the shredder column and proceed with step 4

4. To the above solution add 350µl of ethanol (70%) and mix thoroughly by pulse vortexing (10-15s).
   Note: Some time after addition of ethanol a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to disaggregate any precipitate by mixing and load all of the precipitate on the column as described in step 5. Do not centrifuge the ethanolic lysate before loading it onto the column in order to avoid pelleting the precipitate.

5. Take a new RNASure® Plant Column and place in collection tube and load the lysate . Centrifuge at 11,000 x g for 30 seconds. Then place the column in new collection tube (2ml). Maximum loading capacity of RNA Sure Plant Columns are 750 µl. Repeat the procedure if larger volumes are to be processed

6. Add 350 µl RDB and centrifuge at 11,000 x g for 1 min to dry the membrane.
   Salt removal will help the following rDNase digest much more effective. If the column outlet has come into contact with the flow-through for any reason, discard the flow-through and centrifuge again for 30 s at 11,000 x g.

7. For on column DNase digestion prepare DNase reaction mixture (by adding 10ul of DNase to 90µl of DNase reaction buffer in a 1.5ml microcentrifuge tube). Mix properly and apply 95µl of it directly onto the centre of silica membrane of the RNASure® Column (provided). Incubate atr room temperature for 15 minutes.

8. To the RNASure® column add 200µl of buffer RWB2 and centrifuge for 30 seconds at 11000 x g discard the filtrate. This step will inactivate the rDNase. Place the column in new collection tube.

9. To the RNA Sure column add 600µl of buffer RWB3 & Centrifuge for 30 Seconds at 11,000 x g. Now discard the filtrate. Transfer the column into a new collection tube.

10. Add 200µl of buffer RWB3 to the column & centrifuge it for 2-3 min at 11000 x g this step will dry the membrane. Place the column in new nuclease free 1.5 ml collection tube (supplied). If for any reason, the liquid level in the collection tube has reached the RNASure® Plant column after centrifugation, discard flow-through, and centrifuge again.

11. Elute the RNA in 60 µl RNase-free H_2O, (supplied) and centrifuge at 11,000 x g for 1 min. If higher RNA concentrations are desired, elution can be done with 40 µl but the yield will decrease slightly.

Supplementary Protocol for rDNase digestion in solution

rDNase digestion in solution

- Although,on column rDNase digestion is very efficient in DNA removal. But in certain application even traces of DNA need to be removed, proceed with rDNase treatment in solution.

Prepare rDNase as per instructions

- Prepare rDNase by adding 10µl of RNase Free rDNAse to 90µl of rDNase Buffer. Add 6µl of it to 60µl of eluted RNA (1/10th volume of extracted RNA).
  Incubate the mixture for 10 min at 37°C.
- Repurify the RNA with a suitable RNA cleanup procedure (see manufacturer's protocol of respective kit) or ethanol precipitation.

Ethanol Precipitation

- To one volume of sample add 1/10th volume of 3M sodium Acetate pH 5.2 and 2.5 volumes of 95-100 % chilled ethanol.
- Incubate 30minutes at - 20 °C or 4°C.
- Centrifuge for 10 min at 11000xg.
- Wash RNA pellet with 70 % ethanol.
- Dry RNA pellet and resuspend RNA in RNase-free H_2O.

TROUBLESHOOTING GUIDE

RNA is Degraded/ No RNA is obtained

Possible cause

- RNase contamination

Suggestion(s)

- Use sterile individually wrapped plastic wares
- Use only sterile, disposable RNase free pipette tips and microcentrifuge tube.
- Wear disposable gloves and keep changing the gloves frequently
- Always use proper microbiological technique
- Maintain RNase free working environment
- Glassware should be oven baked for at least 2 hours at 25 °C before use

Possible cause

- Poor RNA Quality or yield

Suggestion(s)

- Follow protocol guidelines of each sample type, buffers preparation etc
- Mix and vortex reagent properly
- Binding of RNA is effective in presence of ethanol only make sure to add ethanol to lysis buffer
- Improper storage of kit components

Possible cause

- Too much starting Material

Suggestion(s)

- Too much of starting material may give low yield. If require buffer RLB1 can be increased.
- Clear homogenate and remove any particulate by use of RNASure® Shredder Columns.

Possible cause

- gDNA Contamination

Suggestion(s)

- rDNase solution not properly applied
- Pipette rDNase solution directly to the center of the silica membrane without wetting the rim

Possible cause

- rDnase not active

Suggestion(s)

- Reconstitute and store lyophilized rDNase as per instruction
- Perform optional DNase digestion step during the sample preparation or after purification.
- Use intron-spanning primer if possible.

Suboptimal performance of RNA in downstream experiments

Possible cause

- Store isolated RNA properly

Suggestion(s)

- Finally eluted RNA properly should always be kept at - 20 °C and for long term storage freeze at -70 °C.

Possible cause

- Presence of ethanol or salt

Suggestion(s)

- Increase centrifugation time during wash steps
- Check flow-through does not touch the column outlet after the second buffer RWB3 wash.
- Use correct order of wash buffer.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure® Plant Mini Kit	50 preps	NP-79105
DNASure® Plant Mini Kit	250 preps	NP-79107
DNASure® Plant Midi Kit	20 preps	NP-78153
DNASure® Plant Maxi Kit	10 preps	NP-78164
DNASure® Blood Mini Kit	50 preps	NP-61105
DNASure® Blood Mini Kit	250 preps	NP-61107
DNASure® Blood Midi Kit	20 preps	NP-61184
DNASure® Blood Maxi Kit	10 preps	NP-61193
DNASure® Blood FastPure Kit	50 preps	NP-62205
DNASure® Blood FastPure Kit	250 preps	NP-62207
SureSpin® Plasmid Mini Kit	50 preps	NP-37105
SureSpin® Plasmid Mini Kit	250 preps	NP-37107
SureSpin® Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin® Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin® Buffer Set*	1	37107-BS
SurePrep® Plasmid Mini Kit	20 preps	NP-15123
SurePrep® Plasmid Mini Kit	100 preps	NP-15125
SurePrep® Plasmid Midi Kit	20 preps	NP-15143
SurePrep® Plasmid Midi Kit	100 preps	NP-15145
SurePrep® Plasmid Maxi Kit	10 preps	NP-15161
SurePrep® Plasmid Maxi Kit	25 preps	NP-15162
SurePrep® Plasmid Mega Kit	5 preps	NP-15183
SurePrep® Plasmid Giga Kit	5 preps	NP-15191

***SureSpin Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

Product Warranty

RNASure® Plant Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the RNASure® Plant Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of RNASure® Plant Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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