IQeasy™ Plus Plant RNA Extraction Mini Kit Handbook

Instruction Manual

For purification of total RNA from Plant samples

Cat. No. 17491 | 50 Columns

Ver 1.0

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IQeasy™ plus Plant RNA Extraction Mini Kit Handbook

Contents

Kit Contents

Label	Description	Contain
Buffer RLE	Lysis Buffer for Protocol I	25 ml
Buffer RB	Binding Buffer for Protocol I	20 ml
Buffer RLD	Lysis Buffer for protocol II	15 ml
Buffer RPB	Binding Buffer for Protocol II	15 ml
Buffer RW1	Washing Buffer A	40 ml
Buffer RW2 (concentrate)[1]	Washing Buffer B	10 ml
Buffer RE	Elution Buffer	20 ml
Sieve Spin Columns	Inserted into a collection tube (2.0 ml tube)	50 columns
gDNA Remover Spin Columns	Inserted into a collection tube (2.0 ml tube)	50 columns
Binding Columns (Red O-ring)	Inserted into a collection tube (2.0 ml tube)	50 columns
Collection Tubes (2.0ml tube)	Additionally supplied	50 tubes

[1] Buffer RLE & RLD contains chaotropic salt can form highly reactive compound.
[2] Buffer RW2 is supplied as concentrates. Add 40ml of ethanol (96 ~ 100%) according to the bottle label before use.

Storage

The IQeasy™ plus Plant RNA Extraction Mini Kit should be stored dry at room temperature(15 ~ 25°C) and is stable for at least 12 months under these conditions

Quality Control

In accordance with iNtRON's ISO-certified Quality Management System, each lot of IQeasy™ plus Plant RNA Extraction Mini Kit is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always should wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please request the appropriate material safety data sheets (MSDS). Do not add bleach or acidic solutions directly to the waste. Buffer RWA contains a chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

Product Warranty and Satisfaction Guarantee

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination

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Product Use Limitations

The IQeasy™ plus Plant RNA Extraction Mini Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. IQeasy™ plus Plant RNA Extraction Mini Kit is developed, designed, and sold for research purpose only. They are not to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. Be careful in the handling of the products.

Technical Assistance

At iNtRON, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the IQeasy™ plus Plant RNA Extraction Mini Kit or iNtRON's products in general, please do not hesitate to contact us.
iNtRON customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques. For technical assistance and more information please call one of the iNtRON Technical Service Departments or local distributors.

Precautions and Safety Information

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable glove. Some buffer contain the chaotrophic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water.
If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Equipment and Reagents to be Supplied by User

IQeasy™ plus Plant RNA Extraction Mini Kit provides almost all reagents for extracting RNA. However, you should prepare some equipments and reagents as follows for a fast and easy extraction. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

IQeasy™ plus Plant RNA Extraction Mini Kit provides almost

- Ethanol (96 ~ 100%)
- Micro-centrifuge
- Micro centrifuge tubes (1.5 ml)
- Other general lab equipments
- β-Mercaptoethanol (14.2M)
- Vortex mixer
- Micro-pipettes and pipette tips

Description

The IQeasy™ plus Plant RNA Extraction Mini Kit provides a fast and easy way to purify total RNA from plant-like samples such as various leaf, stem, root, and seed. This kit is especially useful for extracting RNA from plant tissue, especially difficult plant tissues (seed-like sample), such as those containing polysaccharide (such as starch), polyphenolics, tannins and resins. Because this product does not use chaotrophic salt (e.g. guanidine hydrochloride, guanidine thiocyanate) and acidic phenol for sample lysis. It greatly reduced the risk of the co-precipitation which is precipitated RNA and inhibitors.
The IQeasy™ plus Plant RNA Extraction Mini Kit allows the fastest processing of multiple samples in less than 20 minutes. Time-consuming and tedious methods such as enzymatic lysis incubation, CsCl step-gradient ultracentrifugation and alcohol precipitation steps, or methods involving the use of toxic substances such as phenol and/or chloroform, are replaced by the IQeasy™ plus Plant RNA Extraction Mini Kit procedure.

Characteristics

- Isolated high-quality RNA is suitable for many gene expression profiling techniques :
  - ✓ cDNA synthesis
  - ✓ Reverse Transcriptase PCR(RT-PCR)
  - ✓ Quantitative PCR (qPCR, qRT-PCR)
  - ✓ Microarray
  - ✓ Northern and slot blotting, RNase nuclease protection
- Advanced GxN technology for rapid and efficient purification within 20 min of total RNA from plant samples.
- Chaotropic salt in lysis buffer inactivates immediately RNase to ensure isolation of intact RNA.

Column Information

- IQeasy™ plus Plant RNA Extraction Mini Kit Spin Column

Column membrane[1]	Silica-based membrane
Spin Column[1]	Individually, in inserted in a 2.0 ml Collection Tube[2]
Loading Volume	Maximum 800 µl
RNA Binding Capacity	Maximum 45 µg
Recovery	85 - 95% depending on the elution volume
Elution Volume	Generally, eluted with 30 – 50 µl of Elution Buffer

1 Do not store the Column packs under completely dried conditions. It may be affected to RNA binding capacity. The Spin Columns are stable for over 2 year under these conditions

2 Additional Collection Tubes (100 ea) are also supplied for your convenient handling.

Troubleshooting Guide

Problem	Problem	Recommendation
DNA contamination in downstream experiments	Tissue has high DNA content	–Try using smaller samples (containing less than 20 µg genomic DNA), or perform DNase digestion of the eluted RNA followed by RNA cleanup
	Trace amounts of genomic DNA may still remain	–No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. IQeasy Kits will, however, remove the vast majority of cellular DNA. gDNA Remover Column helps to further reduce genomic DNA contamination but trace amounts of genomic DNA may still remain, depending on the amount and nature of the sample.
Sub-optimal performance of RNA in downstream applications	Carryover of ethanol or salt	–Do not let the flow-through touch the column outlet after the washing steps. Be sure to centrifuge at the appropriate speed for the respective time in order to remove Buffer RW2 completely.
	Isolated RNA not stored properly	–Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20°C, for long term storage freeze at -70°C.

Related Products

Product	Application	Cat. No.
IQeasy™ plus CTB RNA Extraction Mini Kit	total RNA extraction from Blood	17321
IQeasy™ plus Blood RNA Extraction Mini Kit	Total RNA extraction from Plant	17331
IQeasy™ plus Viral DNA/RNA Extraction Kit	Viral DNA/RNA extraction from biological samples	17153
ONE-STEP RT-PCR PreMix Kit	One-step RT-PCR premix, solution type	25131
Power cDNA Synthesis Kit	cDNA synthesis	25011
SiZer™-100 DNA Marker Solution	100bp DNA size marker	24073
SiZer™-1000 DNA Marker Solution	1kb DNA size marker	24074
RNase WiPER™	DNA/RNase remover	21131

Troubleshooting Guide

Problem	Problem	Recommendation
Low RNA yield or no RNA	Perform a mixing experiment	–The purpose of a mixing experiment is to determine whether low RNA yield is due to inadequate tissue disruption, or to the effects of contaminants, such as polysaccharides or phenolics. The strategy in this experiment is to compare yields from an "easy" reference tissue, to yields when the reference tissue is mixed with the problem tissue.
	Too much starting material	–Overloading the spin column significantly reduces RNA yield. Reduce the amount of starting material
	RNA still bound to spin column membrane	–Repeat RNA elution, but incubate the spin column on the bench top for 10 min with RNase-free water before centrifuging.
	Inadequate tissue disruption	–If fresh tissue was used, try snap-freezing and powdering the tissue in liquid nitrogen before disrupting. –To distinguish between low yield due to inadequate disruption versus problems due to release of RNases or other contaminants, perform the mixing experiment described above.
	Tissue has low RNA content	–Some plant tissues have a low RNA content. For example, Arabidopsis has been reported to yield consistently low amounts of RNA from all tissues, and root tissue is reported to have lower-than-average RNA content. Generally, mature leaf tissue has a lower RNA content than young leaves.
	Tissue has high levels of RNases, phenolics, or other contaminants	–To obtain good yields of high quality RNA from problematic tissues such as pine needles or mature cotton tissues, a more rigorous RNA isolation method may be required; for example, ultracentrifugation through cesium chloride. To distinguish between low yield due to contaminants, versus low yield due to inadequate tissue disruption, perform the mixing experiment described above.
RNA is degraded / no RNA obtained	RNase contamination	–Create an RNase-free working environment. Change gloves frequently. Use RNase-free, sterile, disposable polypropylene tubes. Keep tubes closed whenever possible during the preparation. Glassware must be baked overnight at 250°C before use.
	Sample material not stored properly	–Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid nitrogen. Samples should always be kept at -70°C. Never allow tissues to thaw before addition of Buffer RLE or Buffer RLD. Perform disruption of samples in liquid nitrogen.

Important Points Before Starting

- Buffer RW2
  Buffer RW2 is supplied as concentrate. Before using for the first time, be sure to add 40 ml of ethanol (96 ~ 100%) to obtain a working solution.
- Centrifugation
  Centrifugation steps are carried out at 4°C and R.T (15 ~ 25°C) in a micro-centrifuge.

Precaution for Preventing RNase Contamination

RNase can be introducing accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working the RNA.

•Always wear disposable gloves. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNase. Practice good microbial technique to prevent microbial contamination.

Sample Homogenization Techniques

Disruption and homogenization can be performed without lysis buffer by keeping the sample submerged in liquid nitrogen before and during disruption on a mortar. Especially hard tissues, such as roots or seeds, are relatively difficult to be disrupted, and therefore be careful to use a mortar in liquid nitrogen. For optimal result, we recommend to keep the disruption time as short as possible.

Recovery of Purified RNA

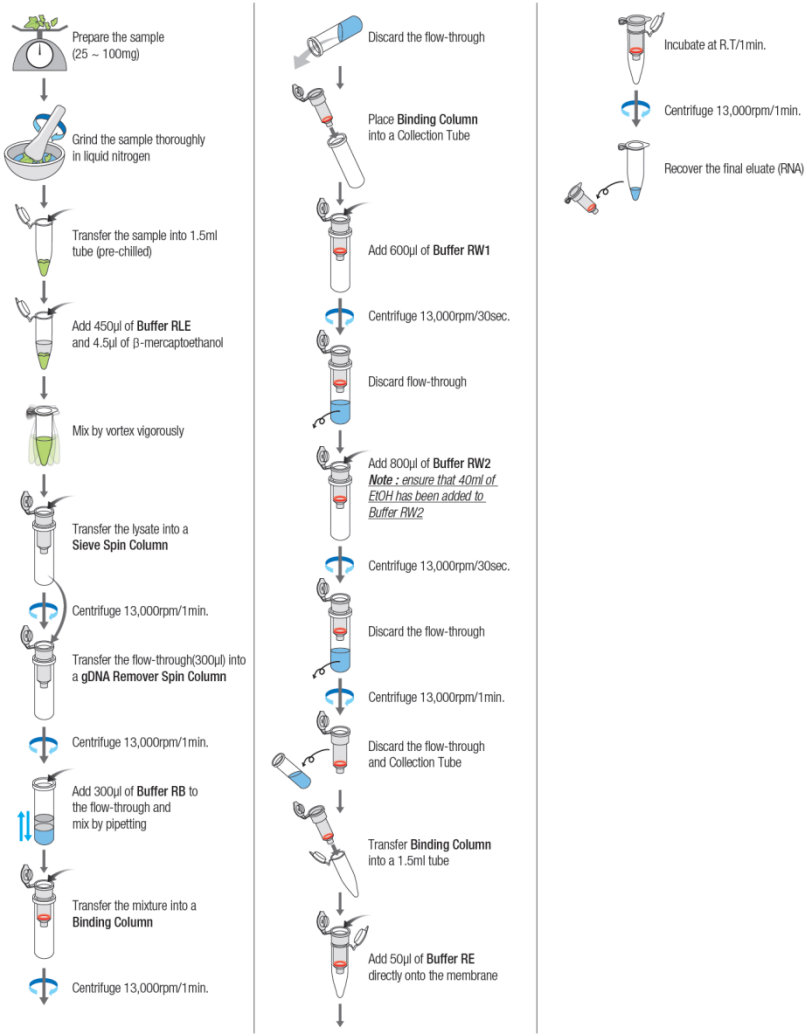
Determination of concentration, yield, and purity RNA yield is determined from the concentration of RNA in elute, measured by absorbance at 260nm. Absorbance readings at 260nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly: for example, the elute containing 4 ~ 40 ng RNA/μl (A_{260} = 0.5 ~ 1.0) should not be diluted with more than 4 volumes of buffer.
Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280nm, or scan absorbance from 220 ~ 320nm (a scan will show if there are other factors affecting absorbance at 260nm). Both RNA and RNA are measured with a spectrophotometer; to measure only RNA, a fluorometer must be used. Purity is determined by calculating the ratio of absorbance at 260nm to absorbance at 280nm. Pure RNA has an A_{260}/A_{280} ratio of 1.8 ~2.0. RNA purified by the IQeasy™ plus Plant RNA Extraction Mini Kit procedure is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Protocols According to the Sample Groups (2 Protocols)

Samples	Protocol Type
Plant Leaf and Seed(*Solanceae*, *Cucurbitaceae*) Plant	Type A Protocol
Seed(*Fabaceae*, *Gramineae*, *Brassicaceae*, etc.)	Type B Protocol

IQeasy™ Plus Plant RNA Extraction Mini Kit procedure

Protocol A : Plant Leaf and Seed (*Solanceae, Cucurbitaceae*)



Technical Information

▪ RNA Q.C data for Microarray

Reliable results in real-time RT-PCR and microarray analysis depend on the quality of the RNA sample. The IQeasy™ plus Plant RNA Extraction Kit provide a high quality of RNA integrity which is determined by nucleic acid analysis, where intact RNA is indicated by a 2:1 ratio of the bands for 28S and 18S rRNA.

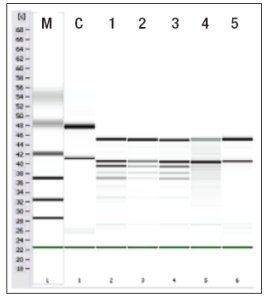


Fig. 2. RNA quality control data for microarray
Total RNA, isolated using the IQeasy™ plus Plant RNA Extraction Mini Kit was run on an Agilent® 2100 bioanalyzer and is displayed here as a gel electropherogram.
Lane M, RNA Ladder Marker; **lane C,** Positive control; **lane 1,** Leaf RNA extraction using easy-spin™ IIp Kit(Cat. No. 17310, iNtRON); **lane 2,** Leaf RNA extraction using Competitor's Kit; **lane 3,** Leaf RNA extraction using IQeasy™ plus Plant RNA Extraction Mini Kit; **lane 4:** Seed RNA extraction using Competitor's Kit; **lane 5,** Seed RNA extraction using IQeasy™ plus Plant RNA Extraction Mini Kit

▪ Optimal Starting Amount of Plant tissue sample

The recovery is increase with sample amount, but using the over-amount of sample hampered high purity of RNA isolation. The recommended sample amount is 50 mg in case of black bean. Generally the optimal range of sample start amount is 25 ~ 100 mg, but the amount is depending on species of sample and sample condition.

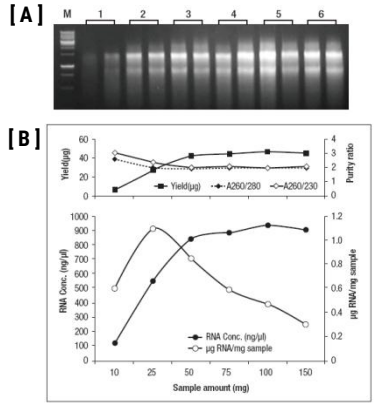


Fig. 3. The influence of sample start amount on RNA isolation
The optimal sample start amount was estimated by electrophoresis and spectrophotometric analysis with black bean (Glycine max) sample. It was shown the optimal start amount around 50 mg of sample.
Panel A, Agarose gel electrophoresis analysis; **Panel B,** Recovery and purity of RNA from different amount of RNA
Lane M, 1 Kb DNA Ladder; **lane 1,** 10 mg; **lane 2,** 25 mg; **lane 3,** 50 mg; **lane 4,** 75 mg; **lane 5,** 100 mg; **lane 6,** 150 mg;

Technical Information

▪ Total RNA Purification form various plant samples

IQeasy™ plus Plant RNA Extraction Mini Kit shows high yield and purity of total RNA extraction from various plant samples.

	RNA Conc (ng/μl)	$A_{260}/_{230}$	$A_{260}/_{280}$	Yield (μg)
Pachira leaf	80	2.01	2.12	4.18
Potato leaf	350	2.13	2.09	18.54
Green pea	660	2.07	2.05	33.22
Black bean	840	1.91	1.91	42.18
Pumpkin seed	160	1.99	2.04	8.16
Watermelon seed	110	2.13	2.07	5.4
Cucumber seed	50	2.17	2.05	2.56
Melon seed	50	1.92	2.03	2.72
Pepper seed	60	2.57	2.17	2.88
Radish seed	350	2.07	2.03	17.56
Spinach seed	340	1.95	2.06	17.16
Tomato seed	110	2.04	2.14	5.7
Rice seed	45	2.08	2.16	2.48
grass seed	40	1.89	2.09	2.2

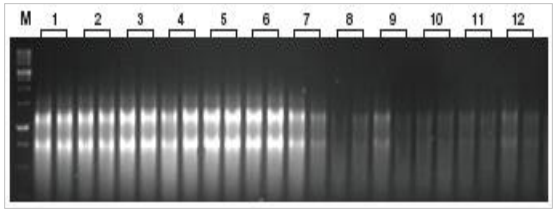
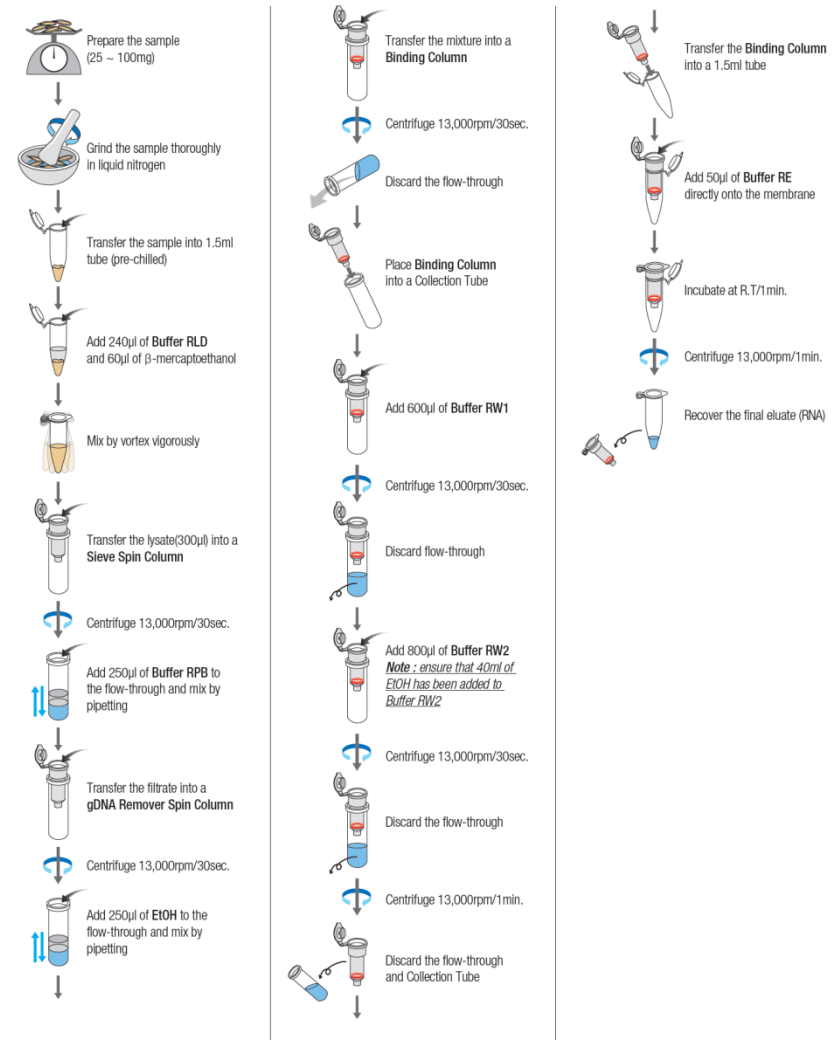


Fig. 1. Results of agarose gel electrophoresis analysis
After eluting total RNA with 50 μl of Buffer RE, each of 5 μl of RNA were used in RNA electrophoresis.
Lane M, 1 Kb Ladder; **lane 1,** Pachira leaf; **lane 2,** Potato leaf; **lane 3,** Pea; **lane 4,** Black bean; **lane 5,** Radish seed; **lane 6,** Spinach seed; **lane 7,** Tomato seed; **lane 8,** Cucumber seed; **lane 9,** Rice seed; **lane 10,** Grass seed; **lane 11,** Pumpkin seed; **lane 12,** Water melon seed

IQeasy™ Plus plant RNA Extraction Mini Kit procedure

Protocol B : Plant Seed (*Fabaceae, Gramineae, Brassicaceae*, etc.)



Protocol A : For Plant Leaf, Seed(*Solanaceae, Cucurbitaceae*)

Refer to the "VISUAL PROTOCOL"

1. **Determine the amount of plant material (25 ~ 100 mg). Do not use more than 100mg.**
   Note : Weighing plant tissue is the most accurate way to determine the amount.
2. **Immediately place the weighed plant tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant plant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 1.5ml micro tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the plant tissue to thaw. Proceed immediately to 'step 3'.**
   Note : RNA in plant tissues is not protected until the tissues are flash-frozen in liquid nitrogen. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.
3. **Add 450 µl of Buffer RLE and 4.5 µl of β-mercaptoethanol (see "Preparing Solution and Equipment Before Use") to a maximum of 100 mg of tissue powder. Vortex vigorously.**
   Note : A short 30 ~ 60sec. vortex mixing may help to disrupt the tissue. However, do not incubate samples with high starch content at elevated temperatures; otherwise swelling of the sample will occur.
4. **Pipette the lysate directly into a Sieve Spin Column placed in a 2 ml collection tube, and centrifuge for 1min. at 13,000rpm.**
5. **Transfer the entire flow-through to the gDNA Remover Spin Columns then centrifuge for 1min. at 13,000rpm.**
   Note : The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.
6. **Add 300 µl of Buffer RB to the flow-through and mix it well.**
7. **After mixing, transfer the mixture to a Binding Column (red color O-ring) in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 1min. at 13,000 rpm. Discard the flow-through.**
   Note : Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.
   Note : The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.
8. **Add 600 µl of Buffer RW1 to the Binding Column. Close the lid gently, and centrifuge for 30sec. at 13,000rpm to wash the spin column membrane. Discard the flow-through.**
   Note : Reuse the collection tube in 'step 8'.
   Note : After centrifugation, carefully remove the Binding Column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.
9. **Add 800 µl of Buffer RW2 to the Binding Column. Close the lid gently, and centrifuge for 30sec. at 13,000rpm to wash the spin column membrane. Discard the flow-through and collection tube.**
   Note : After centrifugation, carefully remove the Binding Column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.
   Note : Ensure that 40 ml of ethanol (EtOH) has been added to Buffer RW2.
10. **Place the Binding Column in a new 2 ml collection tube (supplied), then centrifuge for 1min. at 13,000rpm for column drying.**
    Note : It is very important to dry the membrane of the spin column since residual ethanol may inhibit subsequent reactions. Following the centrifugation, remove carefully the spin column from the collection tube without contacting with the flow through, since this will result in carryover of ethanol.
11. **Place the spin column into a new 1.5ml tube (not supplied), and 50 µl of Buffer RE directly onto the membrane. Incubate for 1min. at room temperature, and then centrifuge for 1min. at 13,000rpm to elute RNA.**
    Note : Elution with 30 µl increases the final RNA concentration, but reduces overall RNA yield conventionally. Alternatively, if you need larger amounts of RNA, eluting with 100 µl increases generally overall RNA yield.

Protocol B : Plant Seed(*Fabaceae, Gramineae, Brassicaceae, etc*)

Refer to the "VISUAL PROTOCOL"

1. **Determine the amount of plant material (25 ~ 100 mg).**
   Note : Weighing tissue is the most accurate way to determine the amount.
2. **Immediately place the weighed tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 1.5 ml micro tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Proceed immediately to 'step 3'.**
   Note : RNA in plant tissues is not protected until the tissues are flash-frozen in liquid nitrogen. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.
3. **Add 240 µl of Buffer RLD and 60 µl of β-mercaptoethanol (see "Preparing Solution and Equipment Before Use") to a maximum of 100 mg tissue powder. Vortex vigorously.**
   Note : A short 30 ~ 60sec. vortex mixing may help to disrupt the tissue. However, do not incubate samples with a high starch content at elevated temperatures, otherwise swelling of the sample will occur.
4. **Pipette the entire lysate directly into a Sieve Spin Column placed in a 2 ml collection tube, and centrifuge for 30sec. at 13,000rpm.**
5. **Add 250 µl of Buffer RPB to the flow-through and mix it well by pipetting.**
6. **After mixing, transfer the mixture to a gDNA Remover Spin Column in a 2 ml collection tube. Close the lid gently, and centrifuge for 30sec. at 13,000rpm.**
   Note : Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.
   Note : The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.
7. **Add 250 µl of absolute ethanol to the flow-through and mix it well by pipetting.**
   Note : When purifying RNA from certain samples, precipitates may be visible after addition of ethanol. This does not affect the procedure.
8. **Transfer the mixture, including any precipitate that may have formed, to a Binding Column (red color O-ring) placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 30sec. at 13,000rpm. Discard the flow-through.**
   Note : Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.
   Note : The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.
9. **Follow the 'Step 8' of Protocol A.**