

peqGOLD Plant RNA Kit

– Instruction Manual –

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Introduction

The peqGOLD Plant RNA Kit provides a convenient and rapid method for the isolation of total RNA from a variety of plant samples. The kit does not require the use of cumbersome or expensive shredding/homogenizing accessories as an attempt to shear DNA in viscous plant lysates. Rather, the method involves a simple and rapid precipitation step for removal of much of the polysaccharides and phenolic compounds commonly found in plant tissues. In combination with HiBind® RNA spin columns, this permits purification of high quality RNA from as much as 200 mg tissue. The system is efficient enough to allow isolation of total RNA from as little as 10 mg of tissue or 100 cells. peqGOLD Plant RNA Kits are ideal for processing multiple plant samples in less than 60 min. Each spin column has a binding capacity of about 100 µg RNA. The need for organic extractions is eliminated, making total RNA isolation fast, safe, and reliable.

RNA purified using the peqGOLD Plant RNA Kit is ready for applications such as RT-PCR*, Northern blotting, poly(A)⁺-RNA (mRNA) purification, nuclease protection assays, and in vitro translation.

Theory

The peqGOLD Plant RNA Kits use the reversible binding properties of the HiBind® matrix, a new silica-based material. This is combined with the speed of mini-column spin technology. A specifically formulated high salt buffer system allows more than 100 µg of RNA molecules greater than 200 bases to bind to the matrix. Cells or tissues are first lysed under denaturing conditions that practically inactivate RNases. Samples are then applied to the HiBind® spin columns to which total RNA binds, while cellular debris and other contaminants are effectively washed out. High quality RNA is finally eluted in DEPC-treated sterile water.

Kit Components

peqGOLD Plant RNA Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6627-00	12-6627-01	12-6627-02
Components			
HiBind [®] Columns	5	50	200
Shredder Columns	5	50	200
2 ml Collection Tubes	15	150	600
RPL Buffer	5 ml	50 ml	120 ml
SP Buffer	2 ml	10 ml	40 ml
RB Buffer	5 ml	30 ml	100 ml
RNA Wash buffer I	5 ml	45 ml	175 ml
RNA Wash buffer II	5 ml	12 ml	45 ml
DEPC-dH ₂ O	1 ml	20 ml	50 ml
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Storage and Stability

peqGOLD Plant RNA Kit components should be stored at room temperature (22 °C – 25 °C). All peqGOLD Total RNA Kit components are stable for at least 12 months from the date of purchase when stored at 22-25 °C. During shipment crystals may form in the RB Buffer. Warm up to 37 °C to dissolve.

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

- ! Whenever working with RNA, always wear one-way gloves to minimize RNase contamination. Use only fresh RNase-free disposable plastic pipette tips when using the supplied reagents.
- ! Work carefully but as quickly as possible during the procedure.
- ! Under cool ambient conditions, crystals may form in the RB Buffer. This is normal and the bottle should be warmed (37 °C) to dissolve the salt before use.
- ! 2-mercaptoethanol (β -mercaptoethanol) is necessary for denaturing RNases and must be added to an aliquot of RPL- and RB Buffer before use. Add 20 μ l of 2-mercaptoethanol to 1 ml of RPL- or RB Buffer. Prepare these mixtures just before use, if possible, however, they can be stored for 1 week at room temperature.
- ! RNA Wash Buffer II is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-6627-00	Add 20 ml 100% EtOH to 5 ml Wash Buffer II
Kit 12-6627-01	Add 48 ml 100% EtOH to 12 ml Wash Buffer II
Kit 12-6627-02	Add 180 ml 100% EtOH to 45 ml Wash Buffer II

Store diluted DNA Wash Buffer at room temperature.

- ! All steps must be carried out at room temperature (22 – 25 °C).

peqGOLD Plant RNA Isolation Protocol

A. Plants

This protocol is suitable for most fresh or frozen tissue samples allowing efficient recovery of RNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to < 200 mg. Best results are obtained with young leaves or needles.

Plant Species	Expected RNA from 1 mg leaf
Arabidopsis sp	0.35 µg
Tobacco leaves	0.60 µg
Mustard leaves	0.34 µg
Maize	0.28 µg
Tomato	0.65 µg

Materials required, but not supplied:

- ! 2-Mercaptoethanol
- ! 100 % Ethanol
- ! Isopropanol
- ! Sterile RNase-free pipet tips and centrifuge tubes

1. Homogenization and lysis

Collect up to 200 mg plant tissue (2 cm² respond to ~ 100 mg) and promptly freeze in a small volume of liquid nitrogen. Grind tissue with a ceramic mortar and pestle under approximately 10 ml of liquid nitrogen.

We recommend starting with 50 mg tissue at first. If results obtained are satisfying increase amount of starting material up to 200 mg.

Wear gloves and take great care when working with liquid nitrogen.

Transfer the suspension into a pre-cooled 15 ml polypropylene tube. If the tube is not pre-cooled (in liquid nitrogen), the suspension will boil vigorously possibly causing loss of tissue. When the liquid nitrogen has completely evaporated, add immediately 600 µl RPL Buffer (completed with 2-Mercaptoethanol) and mix by vortexing until all clumps are dissolved. Pipet the lysate directly into a Shredder Column placed in 2ml collection tube. Centrifuge at 13,000 x g for 5 min at room temperature.

Samples should not be allowed to thaw before RPL Buffer/2-mercaptoethanol is added. Vortex vigorously to make sure that all clumps are dispersed. RNA cannot be effectively extracted from clumped tissue.

For homogenization, you may also use disposable pellet pestles. However, the above methods for disrupting plant tissue cannot be replaced by mechanical homogenizers.

2. Removal of polysaccharides (Optional)

Carefully transfer the supernatant of the flow-through fraction to a new 1.5ml microfuge tube and add 140 μ l SP Buffer and vortex thoroughly to mix. Centrifuge at 10,000 x g for 10 min. Carefully transfer cleared lysate to a new microcentrifuge tube making sure not to disturb the pellet or transfer any debris. Add one volume isopropanol and vortex to precipitate RNA.

In most cases 600 μ l supernatant can easily be removed. This will require 600 μ l isopropanol. Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

This step removes much of the polysaccharide content and improves spin-column performance by increasing RNA binding capacity in the steps that follow.

Immediately centrifuge for 2 min at 10,000 x g to pellet RNA. A longer centrifugation does not improve yields.

Pour off the supernatant making sure not to dislodge the RNA pellet. Invert the microfuge tube on a paper towel for 1 min to allow residual liquid to drain. Drying the pellet is not recommended.

3. Load and bind

Add 100 μ l of pre-heated (65 °C) sterile DEPC-dH₂O (supplied) and vortex to resuspend the pellet. A brief incubation at 65 °C may be necessary to effectively dissolve the RNA.

Add 350 μ l RB Buffer/2-mercaptoethanol first, followed by 250 μ l absolute ethanol. Vortex thoroughly to mix. This will adjust binding conditions prior to loading the HiBind® RNA column.

Place a HiBind® RNA spin column into a fresh 2 ml collection tube (supplied) and apply the entire sample, including any precipitates that may form, to the spin column. Centrifuge the spin column / collection tube assembly at 10,000 x g for 30 sec. Discard the flow-through liquid and place the spin column back into the collection tube.

4. Wash I

Add 750 μ l RNA Washbuffer I to the column and centrifuge the spin column / collection tube assembly for 30 sec at 10,000 x g. Place the spin column in a fresh 2 ml collection tube (supplied). Discard the flow-through liquid and the used collection tube.

5. Wash II

Add 500 μ l completed RNA Washbuffer II to the column and centrifuge the spin column / collection tube assembly for 30 sec at 10,000 x g. Discard the flow-through liquid. Repeat this wash step using the same collection tube and discard the flow-through liquid.

6. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your RNA in the collection tube used in step 5 and centrifuge for 1 min at 10,000 x g to dry the column matrix. This step is essential to remove ethanol from the column.

7. Elution

Place the HiBind® spin column (step 6) into a fresh 1.5 ml microcentrifuge tube. Add 50 - 100 µl (depending on the desired final concentration of RNA) sterile DEPC-dH₂O directly to the binding matrix in the spin column and centrifuge for 1 min at 10,000 x g to elute RNA.

A second elution may be necessary if the expected yield of RNA is >50 µg. Alternatively, RNA may be eluted with a higher volume of water. While additional elution increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution. Pre-heating DEPC-dH₂O to 70 °C before adding to the spin column and incubating the spin column for 5 min at room temperature before centrifugation may increase yield.

B. Arthropods

The exoskeleton of arthropods poses the same problems as encountered with many plant specimens. Pigments and polysaccharides often co-purify with nucleic acids and interfere with downstream applications. The peqGOLD Plant RNA Kit includes a simple isopropanol precipitation step that improves RNA quality and column performance when difficult samples are processed.

Materials required, but not supplied:

- ! 2-Mercaptoethanol
- ! 100 % Ethanol
- ! Isopropanol
- ! Sterile RNase-free pipet tips and centrifuge tubes

1. Homogenization and lysis

Freeze and grind up to 100 mg arthropod tissue under liquid nitrogen. Grind tissue completely to obtain a fine homogenous powder. Immediately add 600 µl RPL Buffer/2-mercaptoethanol and vortex carefully. Pipet the lysate directly into a Shredder Column placed in 2ml collection tube. Centrifuge at 13,000 x g for 5 min at room temperature.

We recommend starting with 50 mg tissue at first. If results obtained are satisfying increase amount of starting material up to 100 mg.

Wear gloves and take great care when working with liquid nitrogen.

Samples should not be allowed to thaw before Buffer RPL/2-mercaptoethanol is added. Vortex vigorously to make sure that all clumps are dispersed. RNA cannot be effectively extracted from clumped tissue.

Proceed with the Plant RNA Protocol from step 2 (page 7).

DNA Contamination

No RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR* or differential display) we suggest that you treat the eluted RNA with RNase-free DNase. Also for RT-PCR, use intron-spanning primers that allow easy identification of DNA-contamination. Using RNA as a template in a control PCR* reaction will also allow the detection of DNA contamination.

Quantitation and storage of RNA

Determine the absorption of an appropriate dilution (10- to 50-fold) of the sample at 260 nm and then at 280 nm.

DEPC-water is slightly acidic and can dramatically lower absorption values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometer analysis.

One A_{260} -unit is about 40 μg RNA/ml. The RNA concentration is calculated as follows:

$$\text{RNA conc. } (\mu\text{g / ml}) = \text{Absorption}_{260} \times 40 \times \text{Dilution Factor}$$

The ratio of $A_{260/280}$ is an indication of nucleic acid purity. Values higher than 1.8 indicates > 90% nucleic acid.

Phenol has an absorption maximum at 275 nm and can interfere with absorption readings of DNA or RNA. However, the peqGOLD Plant RNA Kit eliminates the use of phenol and avoids this problem.

Store RNA samples at $-70\text{ }^{\circ}\text{C}$ in sterile DEPC- dH_2O . Under such conditions RNA prepared with the peqGOLD system is stable for at least one year.

RNA Quality

It is highly recommended to determine the RNA quality prior to further applications. Denaturing agarose gel electrophoresis and ethidium bromide staining can best assess the quality of RNA. Two sharp bands should appear on the gel. These are the 28S and 18S ribosomal RNA bands. If these bands smear towards lower molecular weight RNA, then the RNA has undergone major degradation during preparation, handling, or storage.

Although RNA molecules less than 200 bases in length do not efficiently bind the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

* PCR is covered by patents owned by F. Hoffmann-La Roche Ltd.

Ordering information

For RNA isolation from cells, tissues and blood:

peqGOLD Plant RNA Kit	12-6627-00	5 Preparations
	12-6627-01	50 Preparations
	12-6627-02	200 Preparations
peqGOLD Bacterial RNA Kit	12-6850-00	5 Preparations
	12-6850-01	50 Preparations
	12-6850-02	200 Preparations
peqGOLD Viral RNA Kit	12-6874-00	5 Preparations
	12-6874-01	50 Preparations
	12-6874-02	200 Preparations
peqGOLD Total RNA Kit (Safety-Line)	12-6834-00	5 Preparations
	12-6834-01	50 Preparations
	12-6834-02	200 Preparations
peqGOLD Total RNA Kit (Classic-Line)	12-6634-00	5 Preparations
	12-6634-01	50 Preparations
	12-6634-02	200 Preparations
peqGOLD Blood RNA Kit (Safety-Line)	12-6814-00	5 Preparations
	12-6814-01	50 Preparations
	12-6814-02	200 Preparations
peqGOLD Blood RNA Kit (Classic-Line)	12-6614-00	5 Preparations
	12-6614-01	50 Preparations
	12-6614-02	200 Preparations

Troubleshooting Tips

Problem	Likely cause	Suggestion
Little or no RNA eluted	RNA remains on the column	<ul style="list-style-type: none"> Repeat elution. Pre-heat DEPC-water to 70° C prior to elution. Incubate column for 10 min with water prior to centrifugation.
	Column is overloaded	<ul style="list-style-type: none"> Reduce quantity of starting material.
Clogged column	Incomplete disruption or lysis of plant tissue.	<ul style="list-style-type: none"> Completely disrupt sample in liquid nitrogen. Increase centrifugation time. Reduce amount of starting material
Precipitated RNA will not dissolve.	High nucleic acid and polysaccharide content.	<ul style="list-style-type: none"> Reduce amount of starting material. Generally it is best to start with 50-100 mg at first. To avoid RNA degradation, do not increase incubation time for resuspension.
Degraded RNA	Source	<ul style="list-style-type: none"> Freeze starting material quickly in liquid nitrogen and store at -70°C without thawing. Follow protocol closely, and work quickly. Make sure that 2-mercaptoethanol is added to Buffer RPL.
	RNase contamination	<ul style="list-style-type: none"> Ensure not to introduce RNase during the procedure. Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	<ul style="list-style-type: none"> Ensure Wash Buffer II has been diluted with 100% ethanol as indicated on bottle. Diluted Wash Buffer II must be stored at room temperature. Repeat wash with Wash Buffer II.
DNA contamination	Co-purification of DNA	<ul style="list-style-type: none"> Digest with RNase-free DNase and incubate at 37°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	<ul style="list-style-type: none"> DEPC-treated water is acidic and can dramatically lower Abs₂₆₀ values. Use TE buffer (pH 8) to dilute RNA prior to spec analysis.