

peqGOLD Total RNA Kit (Classic Line)

– Instruction Manual –

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Introduction

peqGOLD Total RNA Kit provides a rapid and easy method for the isolation of up to 100 µg of total RNA from eukaryotic cells and tissues. The kit allows single or multiple, simultaneous processing of samples in less than 30 min. Normally, up to 1×10^7 cells or 40 mg tissue can be used in a single experiment. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation or precipitation with isopropanol or LiCl are eliminated. While this kit may be used for isolation of RNA from whole blood, we recommend to use the peqGOLD Blood RNA Kit (product # 12-6614) as it is specifically designed for effective hemolysis and hemoglobin removal and gives higher RNA yields.

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

peqGOLD Total RNA Kits are available as S- or C-line columns (Safety-Line, # 12-6834-xx or Classic-Line, # 12-6634-xx). S-line columns have a slender form and a tube-like outlet at the bottom, ensuring that all positions in centrifuges and vacuum manifolds can be occupied. Lids close the columns tightly and avoid cross-contamination. C-line columns have a bigger diameter and a flat bottom, allowing higher sample volumes to be processed. These columns possess no lid.

Theory

The peqGOLD Total 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 Total RNA Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6634-00	12-6634-01	12-6634-02
Components			
HiBind® Columns	5	50	200
Shredder Columns	5	50	200
2 ml Collection Tubes	15	150	600
TRK Lysis Buffer	5 ml	50 ml	170 ml
RNA Wash buffer I	5 ml	40 ml	200 ml
RNA Wash buffer II	5 ml	24 ml	2 x 50 ml
DEPC-dH ₂ O	1 ml	5 ml	20 ml
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Storage and Stability

peqGOLD Total 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 TRK Lysis 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 TRK Lysis Buffer. This is normal and the bottle should be warmed (37 °C) to dissolve the salt before use.
- ! 2-mercaptoethanol (β -mercaptoethanol) is necessary in denaturing RNases and must be added to an aliquot of TRK Lysis Buffer before use. Add 20 μ l of 2-mercaptoethanol to 1 ml of TRK Lysis Buffer. Prepare this mixture just before use, if possible, however, it 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 12-6634-00	Add 20 ml 100% EtOH to 5 ml Wash Buffer II
Kit 12-6634-01	Add 96 ml 100% EtOH to 24 ml Wash Buffer II
Kit 12-6634-02	Add 200 ml 100% EtOH to 50 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 Total RNA Isolation Protocol

A. Eucaryotic cells and tissue

Materials required, but not supplied:

- ! β -Mercaptoethanol
- ! 100 % Ethanol
- ! 70 % Ethanol in sterile DEPC-dH₂O
- ! Sterile RNase-free pipet tips and centrifuge tubes

1. Homogenization and lysis

a. Tissue

Excise tissue (~ 40 mg, 3 mm³) 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.

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 400 μ l TRK Lysis Buffer (completed with β -Mercaptoethanol). Transfer the lysate directly into a Shredder Column placed in 2ml collection tube. Centrifuge at .12,000 x g for 5 min at room temperature. Transfer the flow-through lysate into a new 1.5 ml tube.

For RNase rich tissues or more than 40 mg tissue, use 600 μ l of TRK Lysis Buffer. However, use no more than 50 mg tissue.

For homogenization, you may also use glass-, teflon- or electric homogenisators. These methods are faster, but results normally in worse results.

b. Monolayer cells

For tissue culture cells grown in monolayer (adherent fibroblasts, endothelial cells, etc.), lyse the cells directly in the culture vessel as follows. Aspirate culture medium completely and add completed TRK Lysis Buffer directly to the cells. Use 800 μ l for T35 flasks or 10 cm dishes, and 400 μ l for smaller vessels. Pipette buffer over entire surface of vessel to ensure complete lysis. Transfer the lysate directly into a Shredder Column placed in 2ml collection tube. Centrifuge at .12,000 x g for 5 min at room temperature. Transfer the flow-through lysate into a new 1.5 ml tube.

This method is preferable to trypsinization followed by washing because it minimizes RNA degradation by nuclease contamination.

c. Suspension culture

For cells grown in suspension cultures, pellet cells at 1,500 rpm (400 x g) for 5 min. Pour off supernatant and add 400 µl completed TRK-Lysis Buffer per 1×10^7 cells. Transfer the lysate directly into a Shredder Column placed in 2ml collection tube. Centrifuge at $.12,000 \times g$ for 5 min at room temperature. Transfer the flow-through lysate into a new 1.5 ml tube.

2. Load and bind

Add an equal volume (400 µl, 600 µl or 800µl) 70% Ethanol to the lysate and mix thoroughly by vortexing. Place a HiBind® RNA spin column in a 2 ml collection tube (supplied) and add the lysate directly to the membrane. Centrifuge the spin column / collection tube assembly at $10,000 \times g$ for 15 sec. Pour off the flow-through liquid.

A precipitate may form on addition of 70% ethanol. Vortex and add the entire mixture to the column. The maximum capacity of the spin column is 800 µl, larger volumes can be loaded successively. However, the total binding capacity of a spin column is 100 µg RNA.

3. Wash I

Add 750 µl RNA Washbuffer I to the column and centrifuge for 15 sec at $10,000 \times g$. Place the spin column in a fresh 2 ml collection tube (supplied). Discard the flow-through liquid and the used collection tube.

4. Wash II

Add 500 µl completed RNA Washbuffer II to the column and centrifuge for 15 sec at $10,000 \times g$. Discard the flow-through liquid. Repeat this wash step and discard the flow-through liquid.

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

Place the HiBind® spin column containing your RNA in the collection tube used in step 4 and centrifuge for 1 min at $10,000 \times g$ to dry the column matrix. This step is essential to remove ethanol from the column.

6. Elution

Place the HiBind® spin column (step 5) 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 $10,000 \times g$ 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. Blood

This method yields adequate RNA for RT-PCR. This protocol has been tested successfully on fresh whole blood treated with all forms of anticoagulants. For more sensitive work we highly recommend the peqGOLD Blood RNA Kit which specifically lyses and removes erythrocytes prior to leukocyte lysis. This eliminates many inhibitors of PCR such as hemoglobin.

Materials required, but not supplied:

- ! RNase free Proteinase K
- ! β -Mercaptoethanol
- ! 100 % Ethanol
- ! Sterile RNase-free pipet tips and centrifuge tubes

1. Lysis

Prepare a Proteinase K solution by preparing 450 μ l TRK Lysis Buffer/ β -mercaptoethanol containing 4 mg/ml Proteinase K. Transfer 150 μ l of blood into a sterile 1.5 ml microcentrifuge tube and add 350 μ l of TRK Lysis Buffer/ β -mercaptoethanol containing 4 mg/ml Proteinase K and vortex for 30 seconds. Incubate at 70°C for 10 minutes. Mix the sample twice by inversion during the incubation. Centrifuge the sample at 10,000 x g for 3 min and transfer 450 μ l supernatant directly into a Shredder Column placed in 2ml collection tube. Centrifuge at 12,000 x g for 5 min at room temperature. Transfer the flow-through lysate into a new 1.5 ml tube. Add 225 μ l of absolute (100%) ethanol to the mixture and vortex for 10 seconds.

2. Load and bind

Place a HiBind® Spin column in a fresh 2 ml collection tube and transfer the sample from step 1 to the column. Centrifuge the spin column / collection tube assembly for 15 sec at 10,000 x g. Discard the flow-through liquid

A precipitate may form on addition of 70% ethanol. Vortex and add the entire mixture to the column. The maximum capacity of the spin column is 800 μ l, larger volumes can be loaded successively. However, the total binding capacity of a spin column is 100 μ g RNA.

3. Wash I

Add 750 μ l RNA Washbuffer I to the column and centrifuge for 15 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 old collection tube.

4. Wash II

Add 500 µl completed RNA Washbuffer II to the column and centrifuge for 15 sec at 10,000 x g. Discard the flow-through liquid. Repeat this wash step and discard the flow-through liquid.

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

Place the HiBind® spin column containing your RNA in the collection tube used in step 4 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.

6. Elution

Place the HiBind® spin column (step 5) 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 elutions 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.

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. A PCR* reaction, which uses the RNA as template, 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 spectrophotometric 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. A value 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 Total 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.

Ordering information

For RNA isolation from cells, tissues and blood:

peqGOLD Total RNA Kit (C-Line)	12-6634-00	5 Preparations
	12-6634-01	50 Preparations
	12-6634-02	200 Preparations
peqGOLD Total RNA Kit (S-Line)	12-6834-00	5 Preparations
	12-6834-01	50 Preparations
	12-6834-02	200 Preparations
peqGOLD Blood RNA Kit (C-Line)	12-6614-00	5 Preparations
	12-6614-01	50 Preparations
	12-6614-02	200 Preparations
peqGOLD Blood RNA Kit (S-Line)	12-6814-00	5 Preparations
	12-6814-01	50 Preparations
	12-6814-02	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 homogenization	<ul style="list-style-type: none"> • Completely homogenize sample. • Increase centrifugation time. • Reduce amount of starting material
Degraded RNA	Source	<ul style="list-style-type: none"> • Freeze starting material quickly in liquid nitrogen. • Do not store tissue culture cells prior to extraction unless they are lysed first. • Follow protocol closely, and work quickly.
	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 Concentrate has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • 1 X Wash Buffer II must be stored and used at room temperature. • Repeat wash with Wash Buffer II.
DNA contamination		<ul style="list-style-type: none"> • Digest with RNase-free DNase and incubate at 37°C for 5 min.
Low Absorption 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 to dilute RNA prior to spectrophotometric analysis.