

peqGOLD Blood RNA Kit (Classic-Line)

– Instruction Manual –

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Introduction

peqGOLD Blood RNA Kits are designed for isolation of total intracellular RNA from up to 1 ml of fresh, whole blood treated with any common anticoagulant such as heparin, EDTA, or acid-citrate-dextrose. One ml of blood typically yields 1–5 µg of total RNA. The procedure completely removes contaminants and enzyme inhibitors making total RNA isolation fast, convenient, and reliable. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or LiCl, are eliminated. The kit is also suitable for isolation of total RNA from cultured cells, tissues, and bacteria, and from RNA viruses. RNA purified using the peqGOLD Blood RNA method is ready for applications such as RT-PCR.

peqGOLD Blood RNA Kits are available as S- or C-line columns (Safety-Line, # 12-6814-xx or Classic Line, # 12-6614-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.

Principle

The peqGOLD Blood RNA Kits use the reversible binding properties of HiBind® matrix, a new silica-based material. This is combined with the speed of mini-column spin technology. Red blood cells are selectively lysed and white cells collected by centrifugation. After lysis of white blood cells under denaturing conditions that inactivate RNases, total RNA is purified in the HiBind® spin column. A specially formulated high salt buffer system allows RNA molecules greater than 200 bases to bind to the matrix. Cellular debris and other contaminants (such as hemoglobin) are effectively washed away and high quality RNA is finally eluted in DEPC-treated sterile water.

Kit Components

peqGOLD Blood RNA Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6614-00	12-6614-01	12-6614-02
Components			
HiBind® Columns	5	50	200
Shredder Columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer ERL	5 ml	50 ml	3 x 50 ml
TRK Lysis Buffer	5 ml	30 ml	125 ml
RNA Wash buffer I	5 ml	40 ml	4 x 50 ml
RNA Wash buffer II	5 ml	12 ml	4 x 12 ml
DEPC-dH ₂ O	1.5 ml	5 ml	20 ml
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Storage and Stability

peqGOLD Blood RNA Kit components should be stored at room temperature (22 °C – 25 °C). All peqGOLD Blood 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.
- ! ERL Buffer is concentrated and has to be diluted with dH₂O :

Kit 12-6614-	Add 45 ml dH ₂ O to 5 ml 10x ERL Buffer
Kit 12-6614-	Add 450 ml dH ₂ O to 50 ml 10x ERL Buffer
Kit 12-6614-	Add 3 x 450 ml dH ₂ O to 3 x 50 ml 10x ERL Buffer

- ! RNA Wash Buffer II is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-6614-00	Add 20 ml 100% EtOH to 5 ml Wash Buffer II
Kit 12-6614-01	Add 48 ml 100% EtOH to 12 ml Wash Buffer II
Kit 12-6614-02	Add 48 ml 100% EtOH to 12 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 Blood RNA Isolation Protocol

A. Fresh Blood

Materials required, but not supplied:

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

Note: After red blood lysis and removal, all other steps must be carried out at room temperature. Work quickly, but carefully.

1. Lysis of erythrocytes

To 1 volume of whole fresh blood (maximum of 1 ml) add 5 volumes of Buffer ERL (diluted). For example add 5 ml Buffer ERL to 1 ml blood. Mix by vortexing.

Incubate for 15 min on ice, mixing by brief vortexing twice. Lysis of red blood cells is indicated when the solution becomes translucent. For blood samples from individuals with an elevated hematocrit or ECR, extend incubation time to 20 min.

Pellet leukocytes by centrifuging at 600 x g for 10 min at 4°C. Completely remove and discard the supernatant containing lysed red blood cells. If a refrigerated centrifuge is not available, centrifuge at room temperature, but quickly complete next step.

2. Wash White Blood Cell Pellet

Wash the white blood cell pellet with 2 volumes of Buffer ERL per volume of whole blood used in Step 1. Thoroughly vortex to resuspend cells.

Centrifuge at 600 x g for 10 min at 4°C. Again, completely remove and discard the supernatant.

Tip: If you used 1 ml of whole blood, wash with 2 ml of Buffer ERL.

3. Lysis of Leucocytes

Add TRK Lysis Buffer/2-mercaptoethanol to the pelleted white blood cells and vortex thoroughly to mix. For <500µl whole blood add 400µl TRK Lysis Buffer. If 0.5 ml-1.0 ml blood was used in Step 1, add 700µl TRK Lysis Buffer.

Samples may be safely stored at -70°C after addition of TRK Lysis Buffer. Vortex or pipet to remove any clumps.

Note: 2-mercaptoethanol is crucial for inactivating endogenous RNases and must be added to an aliquot of TRK Lysis Buffer. Add 20µl 2-mercaptoethanol per 1 ml of TRK Lysis Buffer. This mixture is stable at room temperature for 2 weeks.

Transfer the cell lysate directly into a Homogenizer Column setting in a 2 ml collection tube (supplied) and centrifuge at maximum speed for 2 minutes. Discard Homogenizer Column and save the homogenized lysate.

Note: if too many cells have been used, the cell lysate will be too viscous to pipet. In this case, divide the sample into two aliquots and adjust the volume of each aliquot to 700µl with TRK Lysis Buffer. Continue the protocol from the step3 with two Homogenizer Columns and two HiBind® RNA Columns.

4. Load and bind

Add an equal volume of 70% ethanol and vortex to mix. A precipitate may form on addition of ethanol, but will not interfere with RNA isolation.

Apply the entire sample (including any precipitate) to a HiBind® RNA column assembled in a 2 ml collection tube (supplied). The maximum capacity of the HiBind® RNA spin cartridge is 750 µl. (Larger volumes can be loaded successively.) Centrifuge at 10,000 x g for 15 seconds. Discard flow-through.

5. Wash I

Wash column with Wash Buffer I by pipetting 750 µl directly into the spin column. Centrifuge at 10,000 x g for 15 seconds and discard the 2 ml collection tube.

6. Wash II

Place column in a new 2ml collection tube (supplied), and add 500µl Wash Buffer II diluted with ethanol. Centrifuge at 10,000 x g for 15 seconds and discard flow-through. Re-use the collection tube in next step.

Note: Wash Buffer II Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

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

Wash column with a second 500µl of Wash Buffer II as described in step 4. Centrifuge and discard flow-through. Then with the collection tube empty, centrifuge the spin cartridge for **1 min at full speed** to *completely* dry the HiBind® matrix.

8. Elution

Transfer the column to a new 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 50-100 µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge 1 min at maximum speed. A second elution may be necessary if >0.5 ml whole blood (>2x10⁶ white blood cells) is used.

Note: No RNA extraction procedure can completely remove genomic DNA. For very sensitive work 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 control PCR reaction containing the RNA as template will also allow detection of DNA contamination.

B. Frozen Blood Samples

Additional materials required:

! RNase-free Proteinase K

Lysis of erythrocytes

Prepare a Proteinase K solution by preparing 450 µl TRK Lysis Buffer/ β -mercaptoethanol containing 4 mg/ml Proteinase K.

Thaw out the frozen blood sample and work quickly to minimize RNA degradation.

Pipette 150 µl of blood into a RNase-free microcentrifuge tube. Add 350 µl of TRK Lysis Buffer/ β -mercaptoethanol containing 4 mg/ml Proteinase K and vortex for 30 seconds to thoroughly mix. Incubate at 70°C for 10 minutes. Mix the sample twice by inversion during the incubation. Centrifuge sample at 10,000 x g for 3 min and transfer 450 µl supernatant to an RNase-free microfuge tube.

Add 225 µl of absolute ethanol to the mixture, vortex for 10 seconds, and proceed to Step 2 (Page 6) of main protocol (addition of sample to RNA HiBind® column/collection tube assembly step).

***Note:** Upon freezing whole blood, both red and white blood cells are lysed. Due to the abundance of contaminants such as hemoglobin, greater than 150 µl frozen blood can not be processed without adversely affecting RNA quality. As leukocytes have a relatively low RNA content, the maximum yield with this protocol is typically < 1 µg. For RT-PCR, a single elution of RNA should be carried out with 30 µl of water to maximize final concentration.*

C. Viral RNA From Acellular Body Fluids (Plasma, Serum, Urine, Etc.)

The following modification of the main Blood RNA protocol is required for optimal binding to the RNA HiBind® matrix.

You will require a stock solution of yeast tRNA to use as carrier.

Prepare a stock solution of yeast tRNA in DEPC-treated dH₂O at 5 mg/ml. Aliquot and freeze at -70°C until required.

Centrifuge no more than 5 ml sample for 20 min at 5,000 x g.

Filter sterilize by passing through a sterile 0.22 µm filter. This will remove any cells, thus avoiding cellular nucleic acid copurification.

Optional: Some specimens may contain very few virions. It may be necessary to concentrate the filtered sample using a centrifugal micro-concentrator. Suitable devices include Centricon™-100 (Amicon, 2 ml, Cat# 4211), Ultrafree™-CL (Millipore, 2 ml, Cat# UFC4 THK 25), and equivalents.

Centrifuge 3-5 ml of sample according to the manufacturer's protocol to obtain a ten- to twenty-fold concentration (final volume 200-300µl).

Pipet 150 µl sample into a 1.5 ml microcentrifuge tube and add 750 µl Buffer TRK Lysis Buffer followed by 5 µl of yeast tRNA. Then add 600 µl absolute ethanol and vortex thoroughly. Immediately proceed to next step.

Note: Add 20 µl 2-mercaptoethanol per 1 ml TRK Lysis Buffer.

Follow the main Blood RNA protocol from the step where you have to transfer the sample onto the Shredder Column.

Use 30-50 µl DEPC-dH₂O for elution to obtain a higher RNA concentration.

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:

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

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.

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

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.