

peqGOLD HP Total RNA Kit (Safety-Line)

– Instruction Manual –

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Introduction

peqGOLD HP Total RNA Kits integrate phenol/guanidine-based lysis and silica-membrane purification of total RNA. TriFast Reagent, included in the kits, is a monophasic solution of phenol and guanidine thiocyanate, designed to facilitate lysis of different sources of samples especially for fatty tissues such as brain and adipose tissue and inhibit RNases.

With the peqGOLD HP Total RNA Kits, all RNA molecules longer than 200 nucleotides are isolated. The procedure provides enrichment for mRNA since most RNAs <200 nucleotides are selectively excluded. The size distribution of purified RNA is comparable to that obtained by centrifugation through a CsCl gradient or cushion, where small RNAs do not sediment efficiently.

RNA purified using the peqGOLD HP 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 HP Total RNA Kits are available as S- or C-line columns (Safety-Line, # 12-6935-xx or Classic-Line, # 12-6936-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 high lysis efficiency of the TriFast Reagent enables use of larger amounts of tissue (up to 100 mg of brain or adipose tissue) with the peqGOLD HiBind RNA Spin Column.

Tissue samples are homogenized in TriFast Reagent. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase.

The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions. The sample is then applied to the HiBind[®] RNA Spin Column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. High-quality RNA is then eluted in 50 - 100 µl of RNase-free water.

Kit Components

peqGOLD HP Total RNA Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6935-00	12-6935-01	12-6935-02
Components			
HiBind [®] Columns S-Line	5	50	200
2 ml Collection Tubes	15	150	600

TriFast Reagent	6 ml	55 ml	220 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

All components except TriFast Reagent in peqGOLD HP Total RNA Kit should be stored at room temperature. TriFast Reagent should be stored at 4°C.

All peqGOLD HP Total RNA Kit components are stable for at least 12 months from the date of purchase when stored at 22-25 °C.

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.
- ! **It is very important to determine the correct amount of starting material before the experiment.** The capacity of the HiBind[®] RNA column is 100µg. For samples contains high amount of RNA, we suggest using 30mg tissue to start. For samples contain lower level RNA, the maximum amount of starting material (100mg) can be used.

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

Kit 12-6935-00	Add 20 ml 100% EtOH to 5 ml Wash Buffer II
Kit 12-6935-01	Add 96 ml 100% EtOH to 24 ml Wash Buffer II
Kit 12-6935-02	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 HP Total RNA Isolation Protocol

Eucaryotic cells and tissue

Materials required, but not supplied:

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

1. Homogenization and lysis

a. Tissue

Homogenize tissue samples in 1ml TriFast™ per 50-100 mg tissue. For efficient lysis use a glass-Teflon or power homogenizer. The sample volume should not exceed 10% of the volume of TriFast™ used for the homogenization.

b. Monolayer cells

Lyse cells directly in a culture dish by addition of 1 ml TriFast™ to a 3.5 cm diameter dish and passing the cell lysate several times through a pipette. The amount of TriFast™ needed is based on the area of the culture dish (1 ml per 10 cm²) and not on the number of cells. An insufficient amount of TriFast™ may result in contamination of the isolated RNA with DNA.

c. Suspension culture

Pellet cells by centrifugation. Lyse cells in TriFast™ by pipetting. Add 1 ml reagent per 5-10 x 10⁶ of cells. Washing cells before the addition of TriFast™ should be avoided as this increases the possibility of RNA degradation.

2. Phase Separation

For dissociation of the nucleoprotein complexes the samples should be kept for 5 minutes at room temperature. Shake samples by hand vigorously after addition of 0.2 ml of chloroform for 15 seconds and keep them for 3-10 minutes at room temperature.

During centrifugation at 12.000 x g for 10 minutes the mixture separates into the lower red (phenol-chloroform phase), the interphase and the colourless upper aqueous phase. RNA is forced exclusively into the aqueous phase whereas DNA and the proteins partition into the interphase and lower phenol phase. The volume of the RNA containing phase is about 60% of the volume of the TriFast™ used for homogenization.

Chloroform used in this experiment should be free of additives like isoamyl alcohol or others.

3. Load and bind

Transfer the upper aqueous phase (around 600ul) into a new 1.5ml centrifuge tube. Add an equal volume 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 650ul of the lysate directly to the membrane. Centrifuge the spin column / collection tube assembly at 10,000 x g for 15 sec. Pour off the flow-through liquid. Add repeat this step by loading the remaining lysate to the column.

A precipitate may form on addition of 70% ethanol. This will not interfere with RNA purification. Vortex and add the entire mixture to the column. The maximum capacity of the spin column is 650 µl, larger volumes can be loaded successively by repeating the loading procedure. However, the total binding capacity of a spin column is 100 µg RNA.

4. Wash I

Add 600 µl RNA Wash buffer 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 used collection tube.

5. Wash II

Add 500 µl completed RNA Wash buffer 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.

6. 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.

7. 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 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.

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 perform on-column DNase I digestion (# 12-1091-01) or 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 A₂₆₀-unit is about 40 µg RNA/ml. The RNA concentration is calculated as follows:

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

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

Store RNA samples at -70 °C in sterile DEPC-dH₂O. 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 rRNA band, may be visible when a large number of cells are used.

Ordering information

For RNA isolation from cells, tissues and blood:

peqGOLD HP Total RNA Kit (C-Line)	12-6936-00	5 Preparations
	12-6936-01	50 Preparations
	12-6936-02	200 Preparations
peqGOLD HP Total RNA Kit (S-Line)	12-6935-00	5 Preparations
	12-6935-01	50 Preparations
	12-6935-02	200 Preparations
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

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.