December 2014

AllPrep® RNA/Protein Handbook

For simultaneous purification of total RNA
and protein from the same animal cell sample

QIAGEN

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Contents

Kit Contents

AllPrep RNA/Protein Kit **Catalog no.** **Number of preps**	**(50)** **80404** **50**
AllPrep Mini Spin Columns (uncolored) (each in a 2 ml Collection Tube)	50
RNeasy® Mini Spin Columns (pink) (each in a 2 ml Collection Tube)	50
Protein Cleanup Mini Spin Columns (uncolored with green cap)	50
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	100
Buffer APL	12 ml
Buffer RLT*	45 ml
Buffer RW1*	45 ml
Buffer RPE† (concentrate)	11 ml
RNase-Free Water	10 ml
Handbook	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

Storage

The AllPrep RNA/Protein Kit should be stored dry at room temperature (15–25°C) and is stable for at least 6 months under these conditions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of AllPrep RNA/Protein Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The AllPrep RNA/Protein Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Technical Assistance

At QIAGEN 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 QIAGEN products. If you have any questions or experience any difficulties regarding the AllPrep RNA/Protein Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN 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 QIAGEN. 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 QIAGEN Technical Service Departments or local distributors (see back cover).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

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

Buffer RLT contains guanidine thiocyanate and Buffer RW1 contains a small amount of guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is split, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Introduction

The AllPrep RNA/Protein Kit is designed to purify total RNA and native proteins simultaneously from a single sample of cultured animal cells. Pure RNA and pure protein are purified from the entire sample, in contrast to other procedures where the biological sample is divided into two before being processed separately.

The AllPrep RNA/Protein Kit allows the parallel processing of multiple samples in less than 40 minutes. Time-consuming and tedious methods such as 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 AllPrep RNA/Protein procedure.

With the AllPrep RNA/Protein procedure, all RNA molecules longer than 200 nucleotides are isolated. The procedure provides an enrichment for mRNA, since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA, and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. The size distribution of the purified RNA is comparable to that obtained by centrifugation through a CsCl cushion, where small RNAs do not sediment efficiently. The purified RNA is ready to use in any downstream application, including:

- RT-PCR and quantitative, real-time RT-PCR*
- Differential display
- cDNA synthesis
- Northern, dot, and slot blot analyses
- Primer extension
- Poly A^+ RNA selection
- RNase/S1 nuclease protection
- Microarrays

Protein purified with the AllPrep RNA/Protein procedure is suited for many protein applications, including:

- SDS-PAGE
- Western blotting
- Enzyme assays
- LiquiChip® assays†

* Visit www.qiagen.com/geneXpression for information on standardized solutions for gene expression analysis, including QuantiTect® Kits and Assays for quantitative, real-time RT-PCR.

† Visit www.qiagen.com/Protein/Assay for information on fast and sensitive multiplex protein assays.

Principle and procedure

The AllPrep RNA/Protein procedure consists of 3 steps: sample lysis, protein purification, and RNA purification.

Sample lysis

Cultured animal cells are lysed and homogenized in Buffer APL. This buffer keeps proteins in their native state and protects RNA from degradation. If desired, the cell lysates can be stored at –70°C for several months until required for RNA and protein purification.

Protein purification

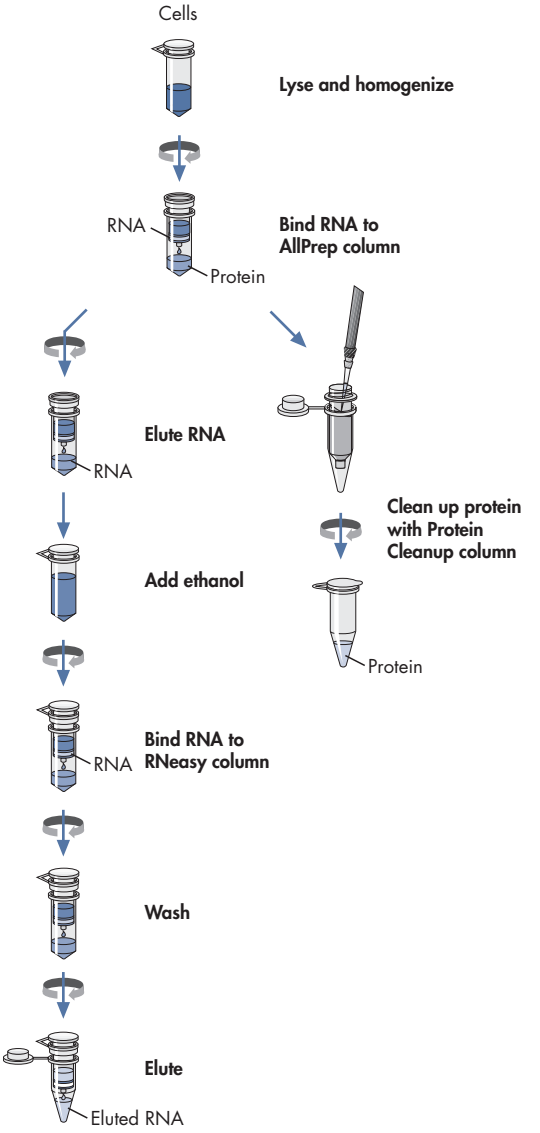
The cell lysate is added to an AllPrep spin column, which is then centrifuged. After centrifugation, RNA is bound to the spin column's silica membrane, while all proteins are in the flow-through. This flow-through is applied to a Protein Cleanup spin column, which is then centrifuged to yield total protein that is free of RNA stabilizing agent and other components of the lysis buffer.

Due to the presence of RNA stabilizing agent in Buffer APL, there may be a reduction in the amount of proteins that have side chains or groups similar to those in nucleic acids (e.g., phosphoproteins). In addition, the exclusion limit of the Protein Cleanup spin column is 1 kDa.

RNA purification

RNA bound to the AllPrep spin column is cleaned up as follows. First, RNA is eluted from the AllPrep spin column using Buffer RLT. The elutate is mixed with ethanol and then centrifuged through an RNeasy spin column. RNA binds to the spin column's silica membrane, and is washed with Buffer RW1 and Buffer RPE before being eluted in RNase-free water.

AllPrep RNA/Protein Procedure



Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Sterile, RNase-free pipet tips
- 96–100% ethanol*
- 70% ethanol* in water
- 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- Ice
- PBS or other physiological salt solution for washing cultured cells
- Equilibration buffer for gel-filtration of the protein fraction; should be compatible with the intended downstream application (e.g., SDS-PAGE)
- Optional: 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M) for addition to Buffer RLT
- Optional: Acetone for precipitation of protein (see Appendix D, page 25)
- Optional: Proteinase and phosphatase inhibitors for stabilizing proteins during lysis (see Appendix E, page 26)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important Notes

Determining the amount of starting material

It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. The maximum amount that can be used is limited by:

- The cell type and its DNA and RNA content
- The volume of Buffer APL required for efficient lysis and the maximum loading volume of the AllPrep and RNeasy spin columns
- The binding capacity of the AllPrep spin column
- The RNA binding capacity of the RNeasy spin column

Refer to Table 1 to make sure that the binding capacity of the spin columns are not exceeded.

Table 1. Specifications of the RNeasy Mini Spin Column

Maximum binding capacity	100 µg RNA
Maximum loading volume	700 µl
RNA size distribution	RNA >200 nucleotides
Minimum elution volume	30 µl

Note: If the binding capacity of the RNeasy Mini spin column is exceeded, yields of total RNA will not be consistent and will be less than expected. If lysis of the starting material is incomplete, yields of total RNA will be lower than expected, even if the binding capacity of the RNeasy Mini spin column is not exceeded.

Note: The RNeasy technology used by the AllPrep RNA/Protein Kit has been modified to enable simultaneous purification of RNA and protein. In some cases, RNA yields may be slightly less than those achieved with RNeasy Kits.

Protocol: Purification of Total RNA and Protein from Animal Cells

This protocol is for use with 12-, 24-, 48-, and 96-well cell-culture plates. If using other formats, please contact QIAGEN Technical Services (see back cover).

Important points before starting

- If using the AllPrep RNA/Protein Kit for the first time, read "Important Notes" (page 11).
- If working with RNA for the first time, read Appendix A (page 20).
- Buffer APL and Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfectants containing bleach. See page 6 for safety information.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at room temperature. During the procedure, work quickly. Cells should be processed immediately after harvesting.
- For storage, quantification, and determination of quality of total RNA, read Appendix B (page 22).

Things to do before starting

- β-ME may be optionally added to Buffer RLT before use to increase RNA yields. We do not recommend using β-ME unless RNA yields from previous purification procedures were low and the troubleshooting guide (page 17) has already been followed. Dispense β-ME in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. The solution is stable for 1 month after the addition of β-ME.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

Procedure

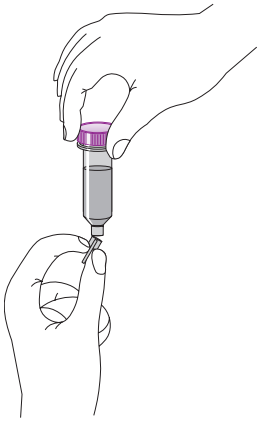
1. **Vortex the Protein Cleanup spin column gently to resuspend the resin.**
2. **Loosen the cap of the Protein Cleanup spin column by a quarter turn.**

   This prevents a vacuum inside the spin column.

3. **Snap off the bottom closure of the Protein Cleanup spin column, and place the spin column in a 2 ml collection tube (supplied).**

   Do not screw off the bottom closure.

Snapping Off the Bottom Closure



4. **Centrifuge for 3 min at 750 x *g* (see Table 4, page 15, to find the equivalent rpm value).**

   A small gap between the gel bed and the wall of the column will form after centrifugation.

5. **Equilibrate the Protein Cleanup spin column by adding 500 µl of equilibration buffer (not supplied), vortexing gently, and centrifuging for 3 min at 750 x *g* (see Table 4, page 15, to find the equivalent rpm value).**

   Choose an equilibration buffer that is compatible with the downstream application(s) in which the purified protein will be used.

6. **Carefully transfer the Protein Cleanup spin column to a clean microcentrifuge tube (not supplied).**

7. **Remove the cell-culture medium. Wash the cells with an appropriate volume of PBS or other physiological salt solution.**

8. **Remove the PBS. Add an appropriate volume of Buffer APL (see Table 2) and incubate for 5 min.**

   Note: Do not incubate on ice during cell lysis. The RNA stabilizing agent will precipitate and be unable to protect the RNA.

Table 2. Recommended Volumes of Buffer APL for Cell Lysis

Cell-culture plate	Volume of Buffer APL per well
12-well plate	200 µl
24-well plate	150 µl
48-well plate	100 µl
96-well plate	50 µl

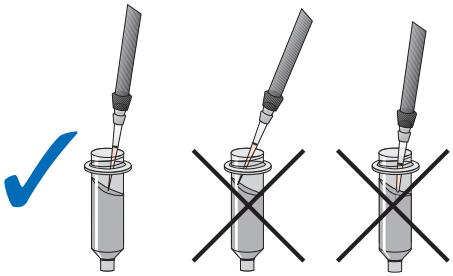
9. **Homogenize by pipetting up and down several times.**
10. **Pipet the lysate into an AllPrep spin column in a 2 ml collection tube and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).**

    The AllPrep spin column contains bound RNA. Keep at room temperature for later use in step 14.

    The flow-through contains total protein. Proceed immediately to step 11. If there will be a slight delay, keep the flow-through on ice.
11. **Slowly (dropwise) pipet the flow-through from step 10 directly onto the center of the slanted gel bed in the Protein Cleanup spin column (see figure below).**

    Important: Pipet slowly so that the drops are absorbed into the gel and do not flow down the gap between the gel bed and the wall of the column. Avoid touching the gel-bed surface with the pipet tip and do not allow the sample or the pipet tip to touch the wall of the column.

Correct Sample Application



12. Centrifuge for 3 min at 240 x *g* or 420 x *g* (see Tables 3 and 4 for details).

Table 3. Centrifuging the Protein Cleanup Spin Column

Volume of Buffer APL per well	Speed (RCF)
200 µl	240 x *g*
150 µl	240 x *g*
100 µl	420 x *g*
50 µl	420 x *g*

Table 4. Centrifugation Conversion Table for Suitable Microcentrifuges*

Microcentrifuge	240 x *g*	420 x *g*	750 x *g*
Eppendorf® Centrifuge 5415C	1700 rpm	2250 rpm	3000 rpm
Eppendorf Centrifuge 5415D	1600 rpm	2130 rpm	2850 rpm
Eppendorf Centrifuge 5417C	1500 rpm	2000 rpm	2700 rpm
Heraeus® Biofuge® 15	1610 rpm	2130 rpm	2800 rpm
Hettich® Mikro 22 R	1490 rpm	1970 rpm	2630 rpm
Hettich Mikro 24-48	1490 rpm	1970 rpm	2630 rpm
Beckman® GS15R	1190 rpm	1570 rpm	2100 rpm
Hettich Mikro EBA12	1500 rpm	2000 rpm	2700 rpm

* The formula for converting relative centrifugal force (RCF) (x *g*) to revolutions per minute (rpm) is rpm = 1000 x $\sqrt{(RCF/1.12r)}$, where *r* is the radius of the rotor in mm.

13. Remove the Protein Cleanup spin column from the microcentrifuge tube.

The flow-through contains purified total protein and is ready for use in downstream applications or for further purification/fractionation.

Note: The purified protein contains buffer components that interfere with some protein quantification methods, including Bradford and Lowry assays. However, protein can be quickly and easily quantified by measuring absorbances at 260 nm and 280 nm (see Appendix C, page 24).* Alternatively, a bicinchoninic acid (BCA) assay can be performed instead.

Note: The functionality of proteins after lysis and cleanup was tested by measuring β-galactosidase activity. The functionality of your protein of interest must be tested according to its properties.

* The AllPrep spin column binds both RNA and DNA. Therefore, absorbance readings of the purified protein are not affected by nucleic acid contamination.

14. **Place the AllPrep spin column in a new 2 ml collection tube (supplied). Add 350 µl Buffer RLT and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).**

15. **Add 1 volume of 70% ethanol (usually 350 µl) to the flow-through. Mix well by pipetting up and down several times.**

    A precipitate may form after the addition of ethanol, but the RNA purification procedure will not be affected.

16. **Add 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column in a 2 ml collection tube. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

    Reuse the collection tube in step 17.

17. **Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.***

    Reuse the collection tube in step 18.

18. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

    Reuse the collection tube in step 19.

    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

19. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

    Reuse the collection tube in step 20.

20. **Centrifuge the RNeasy spin column for 1 min at full speed to remove residual wash buffer.**

    Recommended: Perform a second dry centrifugation: place the spin column in a new 2 ml collection tube (not supplied), discard the old collection tube with the flow-through, and centrifuge at full speed for 1 min.

    Note: It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Dry centrifugation ensures that no ethanol is carried over during RNA elution.

21. **Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute RNA.**

* Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 6 for safety information.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or molecular biology applications (see back cover for contact information).

		Comments and suggestions
Proteins do not perform well in downstream applications		
a)	Incomplete removal of RNA stabilizing agent	Make sure the centrifugation speed for the Protein Cleanup spin column is not higher than that recommended for the amount of lysis buffer used. Be sure to apply the protein-containing flow-through correctly to the Protein Cleanup spin column (see step 11 of the protocol). It is particularly important to avoid protein flowing down the gap between the gel bed and the wall of the column.
b)	Buffer components still present in the purified protein	Different lysates may behave differently. To improve removal of buffer components, reduce the centrifugation speed for the Protein Cleanup spin column. This may result in reduced flow-through volumes and, as a result, reduced protein yields.
c)	Protein degraded	Although protein degradation during cell lysis should have little or no effect on downstream applications, this cannot be completely excluded. Add proteinase and phosphatase inhibitors to Buffer APL to prevent protein degradation (see Appendix E, page 26).
d)	Protein concentration low	The recommended volumes of Buffer APL (see Table 2, page 14) must be used in order to achieve complete cell lysis. Concentrate the protein by following the protocol in Appendix D (page 25) for acetone precipitation of protein. **Important**: Remove RNA stabilizing agent using the Protein Cleanup spin column before performing acetone precipitation (steps 11–13 of the protocol).

	Comments and suggestions
Low RNA yield	
a) RNA still bound to RNeasy membrane	Repeat RNA elution, but incubate the RNeasy spin column on the benchtop for 10 min with RNase-free water before centrifugation.
b) Ethanol carryover	Be sure to dry the RNeasy spin column membrane by performing the two dry centrifugations described in step 20 of the protocol. After the second centrifugation, carefully remove the spin column from the collection tube so that the spin column does not touch the flow-through, which contains ethanol.
Low or no recovery of RNA	
RNase-free water incorrectly dispensed	Add RNase-free water to the center of the RNeasy spin column membrane to ensure maximum RNA elution during the subsequent centrifugation.
Low A_{260}/A_{280} value obtained in RNA quantification	
Incorrect buffer used	Use 10 mM Tris·Cl, pH 7.5, not RNase-free water, to dilute RNA samples before measuring their absorbance at 260 nm and 280 nm (see Appendix B, page 22).
RNA degraded	
a) RNase contamination	Although all buffers (except Buffer APL) have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be sure not to introduce any RNases during the procedure or later handling (see Appendix A, page 20). Do not put RNA samples into a vacuum dryer that has been used in DNA preparations where RNases may have been used.
b) No RNA protection during cell lysis	RNA stabilizing agent was precipitated during cell lysis. Ensure that Buffer APL is at 15–25°C.

RNA does not perform well in downstream applications

a)	Salt carryover during elution	Ensure that Buffer RPE is at 15–25°C.
b)	Ethanol carryover	Be sure to dry the RNeasy spin column membrane by performing the two dry centrifugations described in step 20 of the protocol. After the second centrifugation, carefully remove the spin column from the collection tube so that the spin column does not touch the flow-through, which contains ethanol.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases. For sensitive downstream applications, the use of filter tips is recommended in order to avoid cross contamination.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see “Solutions”, page 21). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for 4 or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol† and allowed to dry.

Solutions

Solutions (water and other solutions)* should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: All buffers in this kit (except Buffer APL) are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Appendix B: Storage, Quantification, and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –20°C or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may be difficult to determine amounts photometrically. Small amounts of RNA can be accurately quantified using an Agilent® 2100 bioanalyzer, quantitative RT-PCR, or fluorometric quantification.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A_{260}=1 → 44 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 23), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 21). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl

Dilution = 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)

A_{260} = 0.2

Concentration of RNA sample = 44 µg/ml x A_{260} x dilution factor
= 44 µg/ml x 0.2 x 50
= 440 µg/ml

Total amount = concentration x volume in milliliters
= 440 µg/ml x 0.1 ml
= 44 µg of RNA

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 22).

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While the the vast majority of cellular DNA will bind to the AllPrep spin column, trace amounts may still remain in the purified RNA, depending on the amount and nature of the sample.

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

* Wilfinger, W. W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

To prevent any interference by DNA in real-time RT-PCR applications, such as with ABI PRISM® and LightCycler® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Assays from QIAGEN are designed for real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible. For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiTect Reverse Transcription Kit provides fast cDNA synthesis with integrated removal of genomic DNA contamination (see ordering information, page 28).

Integrity of RNA

The integrity and size distribution of total RNA purified with RNeasy spin columns can be checked by denaturing agarose gel electrophoresis and ethidium bromide* staining or by using an Agilent 2100 bioanalyzer. The respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S RNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during preparation.

Appendix C: Quantification of Protein

Spectrophotometric quantification

Protein purified using the AllPrep RNA/Protein Kit is free of nucleic acids, since all nucleic acids are removed using the AllPrep spin column. Therefore, the concentration of the purified protein can be easily determined by measuring the absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) in a spectrophotometer:†

$$\text{Protein concentration (mg/ml)} = (1.55 \times A_{280}) - (0.76 \times A_{260})$$

To ensure significance, A_{260} and A_{280} readings should be greater than 0.15.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

† Warburg, O. and Christian, W. (1941) Isolierung und Kristallisation des Gärungsferments Enolase. Biochem. Z. **310**, 384.

Other quantification methods

Protein purified using the AllPrep RNA/Protein Kit can be directly used with the bicinchoninic acid (BCA) assay to estimate the amount of total protein.

Note: Inhibitors or other compounds (see Appendix E, page 26) added after protein cleanup may interfere with the BCA assay. Please check each compound for possible interference.

Other methods for protein quantification, including Bradford and Lowry assays, are not recommended without further cleanup of the purified protein (e.g., by acetone precipitation, dialysis, or ultrafiltration modules).

Appendix D: Acetone Precipitation of Protein

In steps 11 to 13 of the protocol on pages 14–15, RNA stabilizing agent and other components of the lysis buffer are removed from protein-containing flow-through by the Protein Cleanup spin column. Some protein applications may require the protein to be concentrated. This can be achieved by carrying out the procedure below, where protein is precipitated using acetone and then resuspended in the desired buffer.

Important: Remove RNA stabilizing agent using the Protein Cleanup spin column before performing acetone precipitation.

Procedure

D1. Add 4 volumes of ice-cold acetone to the purified protein from the Protein Cleanup spin column. Incubate for 15 min on ice.

D2. Centrifuge for 10 min at 12,000 x *g* in a precooled microcentrifuge at 4°C. Discard the supernatant and air dry the pellet.

Do not overdry the pellet as this may make it difficult to resuspend.

D3. Resuspend the pellet in the buffer used in the downstream application.

Appendix E: Proteinase and Phosphatase Inhibitors for Buffer APL

With the AllPrep RNA/Protein Kit, protein degradation during cell lysis should have little or no effect on downstream applications. However, this cannot be completely excluded. If downstream applications are affected by protein degradation, add proteinase and phosphatase inhibitors to Buffer APL.

Since some inhibitors may be partly removed by the Protein Cleanup spin column, wait until you are ready to start your downstream application before performing protein cleanup (the protein-containing flow-through should be stored at –80°C in the meantime). Alternatively, add the inhibitors again after carrying out protein cleanup.

Table 5. Proteinase Inhibitors for Buffer APL*

Proteinase inhibitor	Concentration of stock solution	Final concentration in Buffer APL
Pefabloc® SC†	100 mM	5 mM
Aprotinin	Available in a	1 µg/ml
Pepstatin A	convenient EDTA-free	1 µg/ml
Leupeptin	mini tablet‡	1 µg/ml

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

† Roche Applied Science, cat. no. 1429876.

‡ Roche Applied Science, cat. no. 11836170001.

Table 6. Phosphatase Inhibitors for Buffer APL

Phosphatase inhibitor	Concentration of stock solution	Final concentration in Buffer APL
β-glycerophosphate	500 mM	5 mM
Sodium orthovanadate	100 mM	1 mM
Sodium fluoride	500 mM	5 mM

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
AllPrep RNA/Protein Kit (50)	50 AllPrep Mini Spin Columns, 50 RNeasy Mini Spin Columns, 50 Protein Cleanup Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80404
Accessories		
Buffer RLT (220 ml)	220 ml Buffer RLT	79216
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
Related products for sample preparation		
AllPrep DNA/RNA Mini Kit — for simultaneous purification of genomic DNA and total RNA from the same animal cell or tissue sample		
AllPrep DNA/RNA Mini Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80204
DNeasy® Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit	50 DNeasy Spin Columns, Proteinase K, Buffer, Collection Tubes	69504
RNeasy Plus Mini Kit — for purification of total RNA from animal cells and easy-to-lyse animal tissues using gDNA Eliminator columns		
RNeasy Plus Mini Kit (50)*	50 RNeasy Mini Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74134

* A wide range of manual and automated kits for RNA purification are available for different sample types, sizes, and throughputs. For details, visit www.qiagen.com/goto/RNA.

Ordering Information

Product	Contents	Cat. no.
Qproteome® Mammalian Protein Prep Kit — for total protein preparations from mammalian cells		
Qproteome Mammalian Protein Prep Kit*	For approximately 100 protein preparations from cultured mammalian cells: Buffer, Reagents, Protease Inhibitor Solution, Benzonase	37901
Related products for downstream applications		
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, RNase-Free Water	205311
QuantiTect SYBR® Green PCR Kit — for quantitative, real-time, two-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green PCR Kit (200)†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204143
QuantiTect SYBR Green RT-PCR Kit — for quantitative, real-time, one-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green RT-PCR Kit (200)†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204243
QuantiTect Probe PCR Kit — for quantitative, real-time, two-step RT-PCR using sequence-specific probes		
QuantiTect Probe PCR Kit (200)†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204343

* A wide range of Qproteome Kits for reproducible and standardized proteomics sample preparation are available. For details, visit www.qiagen.com/Protein.

† Larger kit size available; see www.qiagen.com.

‡ Visit www.qiagen.com/GeneGlobe to search for and order primer sets or primer–probe sets.

Ordering Information

Product	Contents	Cat. no.
QuantiTect Probe RT-PCR Kit — for quantitative, real-time, one-step RT-PCR using sequence-specific probes		
QuantiTect Probe RT-PCR Kit (200)*†	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204443
QuantiTect Multiplex PCR Kits — for quantitative, multiplex, real-time, two-step RT-PCR using sequence-specific probes		
QuantiTect Multiplex PCR Kit (200)*†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (with ROX dye), 2 x 2 ml RNase-Free Water	204543
QuantiTect Multiplex PCR NoROX Kit (200)*†§	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (without ROX dye), 2 x 2 ml RNase-Free Water	204743
QuantiTect Multiplex RT-PCR Kits — for quantitative, multiplex, real-time, one-step RT-PCR using sequence-specific probes		
QuantiTect Multiplex RT-PCR Kit (200)*†‡	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (with ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204643
QuantiTect Multiplex RT-PCR NR Kit (200)*†§	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (without ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204843

* Larger kit size available; see www.qiagen.com.

† Visit www.qiagen.com/GeneGlobe to search for and order primer–probe sets.

‡ Recommended for ABI PRISM and Applied Biosystems® cyclers.

§ Recommended for all other cyclers.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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