omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E-Z 96 Total RNA Kit

R1034-00	1 x 96 preps
R1034-02	12 x 96 preps

Manual Date: January 2024
Revision Number: v4.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek

E-Z 96 Total RNA Kit

Table of Contents

Manual Date: January 2024
Revision Number: v4.0

omega BIO-TEK
innovations in nucleic acid isolation

Product Description

E-Z 96 Total RNA Kits are designed for isolation of cellular RNA from up to 5 x 10^5 cultured cells. This kit allows single or multiple, simultaneous processing of samples in less than 60 minutes. 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.

RNA purified using the E-Z 96 Total RNA method is ready for applications such as RT-PCR*, qPCR*, differential display, microarrays, etc.

The E-Z 96 Total RNA Kits use reversible binding properties of HiBind® matrix, a silica-based, time-saving spin technology material. Utilizing centrifugation or a vacuum manifold, the system allows multiple samples to be processed simultaneously. The sample is lysed under highly denaturing conditions to inactivate RNases while the intact RNA is protected from degradation. After adjusting the binding conditions, the samples are transferred to the E-Z 96 RNA Plate. With a brief centrifugation or vacuum, the samples pass through the plate and the RNA binds to the HiBind® matrix. After three wash steps, purified RNA is eluted with Nuclease-free Water.

New in this Edition:

January 2024:
- The 4x96 prep pack size (R1034-01) has been discontinued and is no longer available to purchase.

November 2018:
- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) has been discontinued and is no longer available to purchase.

August 2013:
- This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product	R1034-00	R1034-02
Purifications	1 x 96	12 x 96
E-Z 96 RNA Plates	1	12
96-well Square-well Plates*	1	4
96-well Racked Microtubes (1.2 mL)	1 x 96	12 x 96
Caps for Racked Microtubes	12 x 8	144 x 8
AeraSeal Film	6	72
TRK Lysis Buffer	20 mL	200 mL
RWF Wash Buffer	60 mL	750 mL
RNA Wash Buffer II	40 mL	2 x 200 mL
Nuclease-free Water	30 mL	250 mL
User Manual	✓	✓

* 96-well Square-well Plates are reusable. See Page 8 for cleaning instructions

Storage and Stability

All of the E-Z 96 Total RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature and away from bright light. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

- Carefully apply the sample or solution to the center of each well of the E-Z 96 RNA Plate. Avoid touching the membrane with pipet tips.

- Optional: 2-mercaptoethanol is key in denaturing RNases and can be added to an aliquot of TRK Lysis Buffer before use. Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. Nuclease-free Water is slightly acidic and can lower A_{260}/A_{280} ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Guidelines for Vacuum Manifold

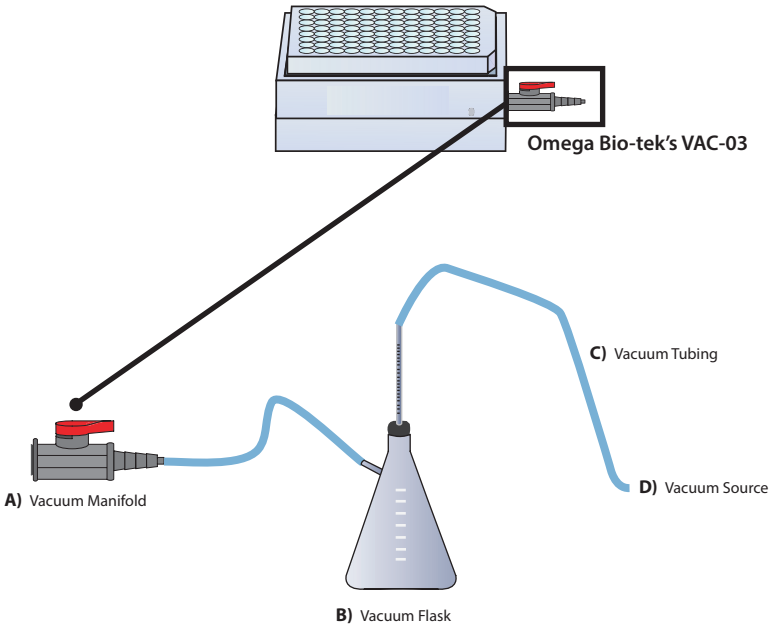
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-03	-200 to -400

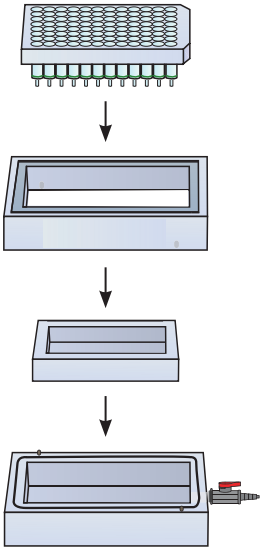
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup



Vacuum Manifold Assembly

RNA Bind & Wash Setup



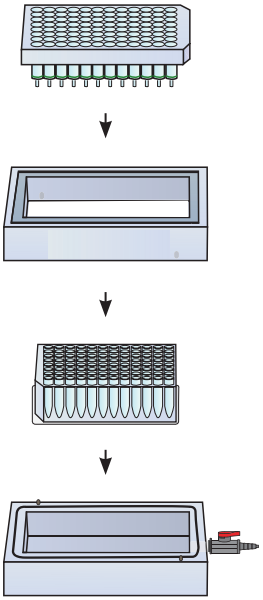
E-Z 96 RNA Plate

Vacuum Manifold Collar

Waste Collection/96-well Square-well Plate

Vacuum Manifold Base

Standard Elution Setup



E-Z 96 RNA Plate

Vacuum Manifold Collar

96-well Racked Microtubes

Vacuum Manifold Base

Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R1034-00	160 mL
R1034-02	800 mL per bottle

Optional: As a preparation step add 20 µL 2-mercaptoethanol (β-mercaptoethanol) per 1 mL TRK Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Cleaning of 96-well Square-well Plates

The 96-well Square-well Plates supplied with this kit are reusable. To avoid cross-contamination, rinse the plates thoroughly with tap water after each use. Soak the plates in 0.2M NaOH/1 mM EDTA overnight. Rinse thoroughly with distilled water and air dry. The 96-well Square-well Plates also can be autoclaved following washing.

Cultured Cells Protocol

Note: All steps must be carried out at room temperature. Work quickly, but carefully.

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 5,000*g* with rotor for 96-well plates
- Multichannel pipette
- Reagent reservoirs for multichannel pipettes
- RNase-free filter pipette tips
- 96-well microplates
- 2 mL 96-well deep-well plates
- Sealing film
- 100% ethanol
- 70% ethanol
- Optional: 2-mercaptoethanol

Before Starting:

- Prepare RNA Wash Buffer II according to the "Preparing Reagents" section on Page 8.
- Optional: Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer.

1. Harvest cells using one of the following methods. Do not use more than 1 x 10^5 cells.

    - For cells grown in suspension:

        1. Determine the number of cells.
        2. Transfer up to 5 x 10^5 cells per well of a 96-well microplate.
        3. Centrifuge at 300 x *g* for 5 minutes.
        4. Aspirate and discard the supernatant.
        5. Proceed to Step 2 on Page 10.

- For cells grown in a monolayer in a multi-well culture plate:

    1. Aspirate and discard the cell culture medium.

    2. Immediately proceed to Step 2 below.

Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate. This will affect the RNA binding conditions of the E-Z 96 RNA Plate and may reduce RNA yield.

2. Add 150 µL TRK Lysis Buffer to each well. Pipet up and down to mix thoroughly.

    Optional: Add 20 µL β-mercaptoethanol per 1 mL TRK Lysis Buffer before use. This mixture can be stored for 4 weeks at room temperature.

    Note: If the multi-well microplate plate has a volume ≤300 µL, use 100 µL TRK Lysis Buffer to limit the total volume in Step 6 to 200 µL.

3. Seal the plate with sealing film (not provided).

4. With the multi-well microplate flat on the bench, shake vigorously end-to-end and side-to-side for a total of one minute.

5. Remove and discard the sealing film.

6. Add one volume 70% ethanol to each well. Pipet up and down 3-4 times to mix thoroughly.

7. Place the E-Z 96 RNA Plate on top of a 96-well Square-well Plate (2.2 mL).

8. Transfer the entire sample from Step 6 (including any precipitates) to each well of the E-Z 96 RNA Plate.

9. Seal the E-Z 96 RNA Plate with AeraSeal Film.

10. Centrifuge at 5,000*g* for 5 minutes at room temperature.

11. Discard the filtrate.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the E-Z 96 RNA Plate eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional DNA removal step is required, please continue to the DNase I Digestion Protocol below. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 12 on Page 12.

E-Z 96 Total RNA Kit - DNase I Digestion Protocol

User Supplied Material:

- DNase I Digestion Set (E1091)

1. For each sample, prepare the DNase I stock solution as follows:

Buffer	Volume per Prep
E.Z.N.A.® DNase I Digestion Buffer	73.5 µL
RNase-free DNase I (20 Kunitz/µL)	1.5 µL
Total Volume	75 µL

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. **Do not vortex the DNase I mixture.** Mix gently by inverting the tube.
- Freshly prepare DNase I stock solution right before RNA isolation.
- Standard DNase buffers are not compatible with on-membrane DNase I digestion. The use of other buffers may affect the binding of RNA to the HiBind® matrix and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

2. Remove and discard the AeraSeal Film.
3. Add 250 µL RWF Wash Buffer to each well.
4. Centrifuge at 5,000*g* for 5 minutes.
5. Discard the filtrate.

6. Add 75 µL DNase I digestion mixture directly onto the surface of each well.

   Note: Pipet the DNase I directly onto the matrix. DNA digestion will not be complete if some of the mixture is retained on the wall of the wells.

7. Let sit at room temperature for 15 minutes.

8. Add 250 µL RWF Wash Buffer to each well.

9. Let sit at room temperature for 2 minutes.

10. Centrifuge at 5,000*g* for 5 minutes.

11. Discard the filtrate.

12. Continue to **Step 16** below.

12. Remove and discard the AeraSeal Film.

13. Add 500 µL RWF Wash Buffer to each well. Seal the E-Z 96 RNA Plate with AeraSeal Film.

14. Centrifuge at 5,000*g* for 5 minutes at room temperature.

15. Remove and discard the AeraSeal Film and discard the filtrate.

16. Add 600 µL RNA Wash Buffer II to each well. Seal the plate with AeraSeal Film.

    Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see Page 8 for instructions.

17. Centrifuge at 5,000*g* for 5 minutes at room temperature.

18. Repeat Steps 15-17 for a second RNA Wash Buffer II step.

19. Centrifuge the empty plate at 5,000*g* for 10 minutes at room temperature.

    Note: It is important to dry the HiBind® matrix before elution. Residual ethanol may interfere with downstream applications.

20. Remove and discard the AeraSeal Film.

21. Place the E-Z 96 RNA Plate onto a set of 96-well Racked Microtubes.

22. Add 50-75 µL Nuclease-free Water to each well. Seal the E-Z 96 RNA Plate with AeraSeal Film.

    Note: Make sure to add water directly onto the center of the HiBind® matrix.

23. Let sit for 3 minutes at room temperature.

24. Centrifuge at 5,000*g* for 5 minutes.

25. Remove and discard the AeraSeal Film.

26. Repeat Steps 22-25 for second elution step.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the plate.
    - Increase the incubation time to 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

27. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store RNA at -70°C.

Vacuum Protocol

Note: All steps must be carried out at room temperature. Work quickly, but carefully. Become familiar with the manifold by reading the manufacturer's instructions before starting this vacuum protocol.

Materials and Equipment to be Supplied by User:

- Vacuum manifold (Product# VAC-03)
- Vacuum source capable of generating a vacuum pressure of -900 mbar
- Centrifuge capable of 5,000*g* with rotor for 96-well plates
- Multichannel pipette
- Reagent reservoirs for multichannel pipettes
- RNase-free filter pipette tips
- 96-well microplates (800 µL)
- 2 mL 96-well deep-well plates
- Sealing film
- Paper towels
- 100% ethanol
- 70% ethanol
- Optional: 2-mercaptoethanol

Before Starting:

- Prepare RNA Wash Buffer II according to the "Preparing Reagents" section on Page 8.
- Optional: Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer.

Note: Please read through previous sections of this manual before proceeding with this protocol. Steps 1-6 from the Cultured Cells protocol should be completed before loading the sample to the E-Z 96 RNA Plate. Instead of continuing with centrifugation, follow the steps below. Do not use more than $1x10^6$ cells for the vacuum protocol.

1. Prepare the vacuum manifold according to manufacturer's instructions. For Omega's VAC-03 manifold, set up the manifold as follows (refer to Page 7 for illustrations):

    a. Place a 96-well Square-well Plate inside the Vacuum Manifold Base.
    b. Place the Vacuum Manifold Collar squarely over the base.

c. Place the E-Z 96 RNA Plate over the Vacuum Manifold Collar.
d. Transfer the samples to the E-Z 96 RNA Plate.
e. Seal the unused wells with sealing film (not provided).

Note: Do not use the AeraSeal Film as this will not provide an adequate seal.

2. Switch on the vacuum source to draw the samples through the wells.

3. Turn off the vacuum.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the E-Z 96 RNA Plate eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional DNA removal step is required, please continue to the DNase I Digestion Protocol below. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 4 on Page 16.

E-Z 96 Total RNA Kit - DNase I Digestion Protocol

User Supplied Material:

- DNase I Digestion Set (E1091)

1. For each sample, prepare the DNase I stock solution as follows:

Buffer	Volume per Prep
E.Z.N.A.® DNase I Digestion Buffer	73.5 µL
RNase-free DNase I (20 Kunitz/µL)	1.5 µL
Total Volume	75 µL

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. **Do not vortex the DNase I mixture.** Mix gently by inverting the tube.

- Freshly prepare DNase I stock solution right before RNA isolation.
- Standard DNase buffers are not compatible with on-membrane DNase I digestion. The use of other buffers may affect the binding of RNA to the HiBind® matrix and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

2. Add 250 µL RWF Wash Buffer to each well.
3. Switch on the vacuum source to draw the RWF Buffer through the wells.
4. Turn off the vacuum.
5. Add 75 µL DNase I digestion mixture directly onto the surface of each well.

   Note: Pipet the DNase I directly onto the matrix. DNA digestion will not be complete if some of the mixture is retained on the wall of the wells.
6. Let sit at room temperature for 15 minutes.
7. Add 250 µL RWF Wash Buffer to each well.
8. Let sit at room temperature for 2 minutes.
9. Switch on the vacuum source to draw the RWF Buffer through the wells.
10. Turn off the vacuum.
11. Continue to **Step 7** below.

4. Add 500 µL RWF Wash Buffer to each well.
5. Switch on the vacuum source to draw the RWF Wash Buffer through the wells.
6. Turn off the vacuum.
7. Add 600 µL RNA Wash Buffer II to each well.

   Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see Page 8 for instructions.

8. Switch on the vacuum source to draw the RNA Wash Buffer II through the wells.

9. Turn off the vacuum.

10. Repeat Steps 7-9 for a second RNA Wash Buffer II step.

11. Remove the E-Z 96 RNA Plate from the vacuum manifold and strike the bottom of the plate on a stack of paper towels. Repeat until there is no liquid released onto the paper towels.

12. Return the E-Z 96 RNA Plate to the manifold.

13. Apply the vacuum for 15 minutes.

14. Turn off the vacuum and disassemble the manifold.

15. Reassemble the manifold as follows (refer to Page 7 for illustrations):

    a. Place a set of 96-well Racked Microtubes into the Vacuum Manifold Base.
    b. Place the Vacuum Manifold Collar squarely over the base.
    c. Place the E-Z 96 RNA Plate over the Vacuum Manifold Collar.

16. Add 50-75 µL Nuclease-free Water to each well.

    Note: Make sure to add water directly onto the center of the HiBind® matrix.

17. Let sit for 3 minutes at room temperature.

18. Apply the vacuum for 5 minutes.

19. Turn off the vacuum.

20. Repeat Steps 16-19 for second elution step.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the plate.
    - Increase the incubation time to 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

21. Remove and discard the sealing film.

22. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store RNA at -70°C.

E-Z 96 Total RNA Clean Up - Centrifugation Protocol

1. Adjust the volume of the RNA samples to 150 µL with RNase-free water.

2. Add one volume 70% ethanol to each sample. Pipet up and down 3-4 times to mix thoroughly.

3. Place a E-Z 96 RNA Plate on top of a 96-well Square-well Plate (2.2 mL).

4. Transfer the entire sample from Step 2 to each well of the E-Z 96 RNA Plate.

5. Seal the E-Z 96 RNA Plate with AeraSeal Film.

6. Centrifuge at 5,000*g* for 5 minutes at room temperature.

7. Discard the filtrate. Remove and discard the AeraSeal Film.

8. Add 800 µL RNA Wash Buffer II to each well. Seal the plate with AeraSeal Film.

   Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see Page 8 for instructions.

9. Centrifuge at 5,000*g* for 10 minutes at room temperature.

10. Remove and discard the AeraSeal Film.

11. Place the E-Z 96 RNA Plate onto a set of 96-well Racked Microtubes.

12. Add 50-75 µL Nuclease-free Water to each well. Seal the E-Z 96 RNA Plate with AeraSeal Film.

    Note: Make sure to add water directly onto the center of the HiBind® matrix.

13. Let sit for 3 minutes at room temperature.

14. Centrifuge at 5,000*g* for 5 minutes.

15. Remove and discard the AeraSeal Film.

Optional: Repeat Steps 12-15 for second elution step.

Note: Any combination of the following steps can be used to help increase RNA yield.

- Heat the Nuclease-free Water to 70°C before adding to the plate.
- Increase the incubation time to 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
- Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

16. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store RNA at -70°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the plate	• Repeat elution • Heat Nuclease-free Water to 70°C prior to elution • Let sit for 5 minutes with Nuclease-free Water prior to elution
	Plate is overloaded	• Reduce the quantity of the starting material
Problem	**Cause**	**Solution**
Degraded RNA	Source	• Do not freeze and thaw sample more than once • Follow protocol closely and work quickly • Add β-ME to TRK Lysis Buffer
	RNase contamination	• Ensure not to introduce RNase during the procedure • Check buffers for RNase contamination
Problem	**Cause**	**Solution**
Problem in downstream applications	Salt carryover during elution	• Ensure RNA Wash Buffer II has been diluted with 100% ethanol as indicated on bottle • RNA Wash Buffer II must be stored at room temperature • Repeat wash with RNA Wash Buffer II
	Inhibitors of PCR	• Use less starting material • Prolong incubation with TRK Lysis Buffer to completely lyse cells
Problem	**Cause**	**Solution**
Clogged well	Incomplete lysis	• Mix thoroughly after addition of TRK Lysis Buffer • Reduce the amount of the starting material
DNA contamination		• Digest with RNase-free DNase and inactivate at 37°C for 5 minutes

Notes:

Notes:

Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek