



## Technical Bulletin

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# MagaZorb® Total RNA Mini-Prep Kit

INSTRUCTIONS FOR USE OF PRODUCT MB2004.



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# MagaZorb® Total RNA Mini-Prep Kit

Please contact Promega Technical Services if you have questions on use of this system.  
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## 1. Description

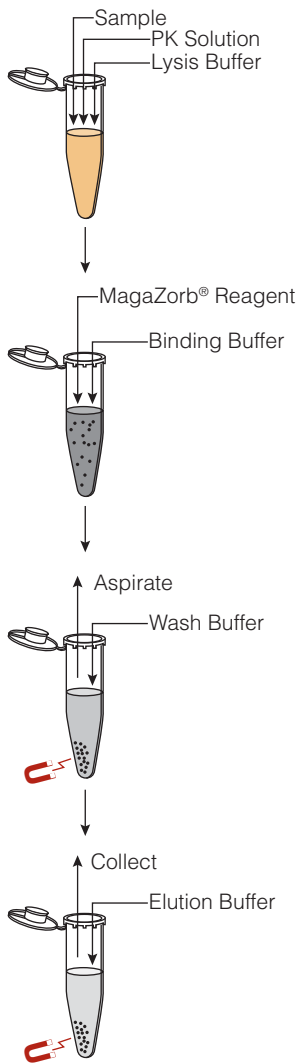
The MagaZorb® Total RNA Mini-Prep Kit<sup>(a)</sup> provides an easy, fast and cost-effective technique for isolating PCR-quality total RNA. Using one simple protocol, a high yield of purified total RNA can be isolated from various sources including whole blood (fresh or citrate-, heparin- or EDTA-treated), buffy coat, leukocytes and tissue (fresh or frozen).

The MagaZorb® Total RNA Isolation Kit does not require an organic solvent, eliminating the need for special storage or waste disposal. All reagents needed are included with the kit—no reagent preparation is required. The MagaZorb® proprietary technology eliminates the need for centrifugation, vacuum filtration or column separation, thereby increasing sample throughput and improving reproducibility. RNA purified by MagaZorb® Total RNA Mini-Prep Kit is ready for downstream applications such as PCR, sequencing or blotting procedures.

The MagaZorb® Total RNA Mini-Prep Kit is based on the specific interaction between nucleic acids and the proprietary magnetizable particles in the presence of specially formulated buffer reagents. This simple but elegant technology is designed so that the binding of nucleic acids is not dependent on chaotropic agents.

RNA in the sample is liberated using Proteinase K (PK) Solution and Lysis Buffer. Released RNA is bound exclusively and specifically to the MagaZorb® Reagent in the presence of the Binding Buffer. The RNA bound to MagaZorb®

particles is captured by a magnet, and contaminants are removed by washing with a single Wash Buffer. The RNA is then eluted from the particles with RNase-Free Water. A schematic representation of the MagaZorb® RNA protocol is shown in Figure 1.



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**Figure 1. Schematic of the MagaZorb® Total RNA Mini-Prep Kit assay.**

## 2. Product Components and Storage Conditions

Product	Size	Cat.#
MagaZorb® Total RNA Mini-Prep Kit	200 preps	MB2004

Cat.# MB2004 contains sufficient reagents for approximately 200 RNA isolations.

Includes:

- 4ml Proteinase K (PK) Solution
- 40ml Lysis Buffer
- 100ml Binding Buffer
- 4ml MagaZorb® Reagent
- 2 × 200ml Wash Buffer
- 40ml RNase-Free Water

**Storage Conditions:** Store the MagaZorb® Total RNA Mini-Prep Kit at room temperature (15–25°C). All components are stable until the date noted on the kit label when stored at room temperature.

## 3. Protocol

### 3.A. Isolation of Total RNA from Whole Blood

#### Materials to Be Supplied by the User

- pipettors (20µl, 100µl and 1,000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- 2ml microcentrifuge tubes
- water bath: 56°C or tight-fitting heating block
- aspiration device
- glass pipets
- optional: end-over-end tube rotator

### 3.A. Isolation of Total RNA from Whole Blood (continued)

#### Notes:

1. The use of clean, disinfected tubes and pipette tips designed for molecular biology applications is recommended.
2. Tube caps should be kept closed at all times during this procedure. Open only for additions, aspirations and eluate collection.
3. MagaZorb® RNA has been optimized for fresh whole blood. Frozen whole blood is not recommended. Fresh whole blood can be stored at 4°C for up to 24 hours before use.
4. Sample volumes can be changed to obtain the desired yields. Please see Section 4.A for details.

### Lysis Protocol

#### Before You Begin

- Bring the samples to room temperature.
  - Check all buffers for precipitates.
  - Gently mix the PK Solution immediately before addition by hand swirling. Do not vortex.
  - Gently mix the Lysis Buffer immediately before addition by hand swirling. Avoid generating bubbles or foam. If precipitate is present, warm at 56°C for 5–10 minutes or until a clear solution is obtained.
  - Mix the sample immediately before addition by pulse-vortexing for 10 seconds or until all the precipitated cells are well dispersed
1. Add 20µl of well mixed PK Solution into a new 2ml microcentrifuge tube at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
  2. Add 200µl of well mixed sample into the microcentrifuge tube directly on top of the PK Solution. Avoid touching the sides of the microcentrifuge tube while pipetting.
  3. Mix gently by manual swirling. Take care not to allow any of the sample/PK solution to be caught in the microcentrifuge tube cap.
  4. Add 200µl of well mixed, clear Lysis Buffer to the microcentrifuge tube.
  5. Mix well by pulse-vortexing until a homogeneous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
  6. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C also can be used.
  7. Remove the tube from 56°C, and dry the outside of the tube with a paper towel. An olive or green color is indicative of complete lysis if whole blood is used as the sample.

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## Binding Protocol

### Before You Begin

- Mix the Binding Buffer immediately before use by inverting or pulse-vortexing.
  - Mix the MagaZorb® Reagent immediately before addition to the tube in Step 10 by vortexing or rigorous hand swirling until the particles are uniformly suspended. If multiple samples are being used, the MagaZorb® Reagent should be mixed between each addition.
8. Add 500µl of the well mixed Binding Buffer to the microcentrifuge tube from the final Lysis step, Step 7.
  9. Mix well by pulse-vortexing until a homogeneous mixture is obtained, usually 15 seconds. This also can be achieved by simply inverting the tube 8-10 times. Tap the tube gently to release any solution caught in the tube cap.
  10. Add 20µl of well mixed MagaZorb® Reagent to the microcentrifuge tube from Step 9 directly into the solution.
  11. Mix by gentle pulse-vortexing, usually 15 seconds.
  12. Incubate at room temperature for 10 minutes while mixing, using an end-over-end tube rotator or occasional manual mixing (every 2 minutes). If a rotator is used, visually ensure that complete mixing is occurring.
  13. Sediment the MagaZorb® particles using a magnetic rack. Sedimentation duration depends on the strength of the magnet. Using the Magnetic Accessories, particles usually sediment within 60-90 seconds. While holding the tubes onto the magnet, invert the magnet 2-3 times to rinse the tube cap with the supernatant.
  14. Remove the supernatant by aspiration. The tube should be tightly pressed against the magnet during the aspiration to ensure the magnetic particles in the tube are tightly attached to the magnet. Remove as much liquid as possible, including any liquid trapped in the tube cap, taking care not to remove any particles.

## Wash Protocol

### Before You Begin

- Mix the Wash Buffer by inverting or swirling.
15. Add 1ml of well mixed Wash Buffer to the tube from Step 14 of the Binding Protocol.
  16. Remove the tube from the magnet, and mix well by inverting the tube several times to ensure the particles are completely dispersed.
  17. Sediment the particles on the magnetic rack as in Step 13 of the Binding Protocol.

### 3.A. Isolation of Total RNA from Whole Blood (continued)

#### Wash Protocol (continued)

18. Remove the supernatant by aspiration as in Step 14.
19. Repeat Steps 15-18 to perform a second wash. The supernatant after the second wash should be very clear. Be sure to remove all liquid by aspiration in the second wash, including any liquid trapped in the tube cap. Take care not to remove any particles. The microcentrifuge tube should be dry, with only the MagaZorb® particles firmly pelleted at the bottom of the tube.

#### Elution Protocol

20. Add 50-100µl, or desired amount, of the well mixed RNase-Free Water to the tube from Step 19.  
**Note:** The standard elution volume for most applications is 50µl. Smaller elution volumes (20µl) also can be used. For smaller elution volumes, efficient mixing of particles is critical in order to obtain the maximum yield of RNA. Manual mixing is recommended for elution volumes of <100µl.
21. Remove the tube from the magnet, then mix gently by inverting and swirling.
22. Incubate the tube for 10 minutes at room temperature using an end-over-end tube rotator or occasional manual mixing (every 2 minutes).
23. Sediment the particles on the magnetic rack as in Step 13.
24. Carefully transfer the supernatant into a clean tube. Avoid collecting particles during the transfer. The transferred supernatant contains the purified RNA.
25. The material is ready for further analysis. If the isolated RNA is not going to be tested on the same day, it should be frozen at -70°C until the time of analysis.

### 3.B. Isolation of Total RNA from Buffy Coat and Leukocytes

#### Materials to Be Supplied by the User

- MagaZorb® Total RNA Mini-Prep Kit
- pipettors (20µl, 200µl, 1,000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block
- microcentrifuge tubes (2ml)
- end-over-end tube rotator (optional)
- aspiration device
- glass pipettes for aspiration
- 0.9% NaCl
- buffy coat preparation (e.g., Accu-Prep™ Kit from Accurate Chemical & Scientific Corp., Cat.# AN 5511)
- optional: RNAlater® (Ambion Cat.# AM7020)

#### Notes:

1. The use of clean, disinfected tubes and pipette tips designed for molecular biology applications is recommended.
2. Tube caps should be kept closed at all times during this procedure. Open only for additions, aspirations and eluate collection.
3. Sample volumes can be changed to obtain the desired yields. Please see Section 4.A for details.

### Lysis Protocol

#### Before You Begin

- Bring the samples to room temperature.
  - Check all buffers for precipitates.
  - Gently mix the PK Solution immediately before addition by hand swirling. Do not vortex.
  - Gently mix the Lysis Buffer immediately before addition by hand swirling. Avoid generating bubbles or foam. If precipitate is present, warm at 56°C for 5–10 minutes or until a clear solution is obtained.
  - Mix the sample immediately before addition by pulse-vortexing for 10 seconds or until all the precipitated cells are well dispersed.
1. Prepare white blood cells (WBC) from 1.5–3ml of fresh whole blood according to standard procedures of the Accu-Prep™ Kit (Accurate Chemical & Scientific Corp., Cat.# AN 5511).

**Note:** Proceed to either Step 2 or Step 3, depending on whether you wish to process immediately or freeze sample for later processing.



### 3.B. Isolation of Total RNA from Buffy Coat and Leukocytes (continued)

#### Lysis Protocol (continued)

2. **To save the sample for later processing:** Resuspend the WBC preparation from Step 1 in 50–100µl of 0.9% NaCl. Mix gently to break the pellet into a completely uniform suspension.

Add 200–400µl of RNAlater® (Ambion Cat.# AM7020) to the preparation from Step 2, and mix gently. The sample can be stored frozen at –70°C until use. Allow only one freeze-thaw cycle. After thawing, proceed to Step 4.

3. **Alternatively, to process the sample immediately:** Resuspend the WBC preparation from Step 1 in 250–500µl of 0.9% NaCl. Mix gently to break the pellet into a completely uniform suspension.

Process immediately.

4. Add 20µl of well mixed PK Solution to a clean 2ml microcentrifuge tube at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
5. Add 300µl of the well mixed WBC preparation from Step 2 or 3 to the microcentrifuge tube directly on top of the PK Solution. Avoid touching the sides of the microcentrifuge tube while pipetting.
6. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
7. Add 200µl of well mixed, clear Lysis Buffer to the microcentrifuge tube.
8. Mix well by pulse-vortexing until a homogeneous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
9. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C also can be used.
10. Remove the tube from 56°C, and dry the outside of the tube with a paper towel.
11. Please refer to Section 3.A for Binding, Wash and Elution protocols.

**Note:** The recommended elution volume for this application is 50µl.

### 3.C. Isolation of Total RNA from Fresh Frozen Tissue

#### Materials to Be Supplied by the User

- pipettors (20 $\mu$ l, 200 $\mu$ l, 1,000 $\mu$ l)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block
- 2ml microcentrifuge tubes
- RNA<sup>later</sup>® solution (Ambion, Cat.# AM7020)
- 1% SDS solution
- tissue grinder (For maximum yield of RNA from a tissue specimen it is essential to mechanically disrupt the tissue prior to RNA isolation. A tissue grinder such as the Pellet Pestle® Cordless Motor tissue grinder (Cat.# 749540-0000) from Kimble/Kontes, Vineland, NJ, or the equivalent, is recommended.)
- aspiration device
- glass pipettes for aspiration
- optional: end-over-end tube rotator

#### Notes:

1. In applications where tissue disruption is not possible or undesirable, customers have reported that overnight lysis of the tissue also achieves desirable results.
2. The use of clean, disinfected tubes and pipette tips designed for molecular biology applications is recommended. Microcentrifuge tubes with longer or sharper conical bottoms are preferred for the homogenization step.
3. Tube caps should be kept closed at all times during this procedure. Open only for additions, aspirations and eluate collection.

### Lysis Protocol

#### Before You Begin

- Check all buffers for precipitates.
- Gently mix the PK Solution immediately before addition by hand swirling. Do not vortex.
- Gently mix the Lysis Buffer immediately before addition by hand swirling. Avoid generating bubbles or foam. If precipitate is present, warm at 56°C for 5-10 minutes or until a clear solution is obtained.
- Keep the tissue sample on ice at all times during Steps 1 and 2.

### 3.C. Isolation of Total RNA from Fresh Frozen Tissue (continued)

#### Lysis Protocol (continued)

1. Create a tissue homogenization solution (THS) by adding 100µl of RNAlater® solution (Ambion) and 100µl of 1% SDS solution for each sample into a new 2ml microcentrifuge tube. If samples >10mg are to be used, increase the amount of THS. Use the THS on the same day it is prepared. The THS will be cloudy but without particulates or precipitation.
2. Add 2.5–10mg of tissue to a clean 2ml microcentrifuge tube.
3. Homogenize the tissue in 200µl THS using a tissue grinder. A uniform suspension should be obtained within 5–10 minutes. Keep the tissue homogenate on ice until you proceed to Step 4.
4. Add 20µl of well mixed PK Solution into the tube from Step 3 at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
5. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
6. Add 200µl of well mixed Lysis Buffer to the microcentrifuge tube.
7. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
8. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C also can be used.
9. Remove the tube from 56°C, and dry the outside of the tube with a paper towel.
10. See Section 3.A for Binding, Wash and Elution Protocols.

**Note:** The recommended elution volume for this application is 50µl.

## 4. Notes and Troubleshooting

### 4.A. Notes and Custom Sample Volumes

1. The MagaZorb® Total RNA Mini-Prep Kit has been optimized for use with fresh whole blood (<24 hours old). Frozen blood samples are not recommended because the total RNA is degraded under this storage condition. Also, blood sample collection plays an important role in the quality of purified total RNA. Extra care should be practiced during blood collection, storage, handling and transportation to avoid possible contamination of the sample.

2. Proper microbiological aseptic technique should always be used when working with total RNA. All plasticware and glassware should be free of RNase and DNase contamination. Keep the tubes closed during the procedure, work quickly and keep the purified RNA on ice until used.
3. Typically >3µg of total RNA is recovered from 1ml of whole blood. The yield is donor-dependent; therefore, higher or lower values may be obtained.
4. To determine the RNA purity using  $A_{260}/A_{280}$ , use 10mM Tris-HCl (pH 7.5), not RNase-free water, to dilute the sample.

### Custom Sample Volumes

The sample volume for the MagaZorb® Total RNA Mini-Prep Kit is highly flexible and easily tailored to meet customer needs. The sample volume can be increased or decreased; the volume of the other components scale up or down linearly. Use the worktable below to calculate the volume of components needed if the desired sample volume is greater or less than suggested in the standard protocol.

**Definition:** SVF = Sample Volume Factor

Determine SVF:  $\frac{(\text{Desired Sample Volume})}{(\text{Standard Sample Volume})} = \text{_____ (SVF)}$

Component Name	Standard Volume × SVF = Customer-Specific Volume
PK Solution	20µl × ____ (SVF) = _____µl (Custom Volume)
Lysis Buffer	200µl × ____ (SVF) = _____µl (Custom Volume)
Binding Buffer	500µl × ____ (SVF) = _____µl (Custom Volume)
MagaZorb® Particles	20µl × ____ (SVF) = _____µl (Custom Volume)
Wash Buffer	1,000µl × ____ (SVF) = _____µl (Custom Volume)
RNase-Free Water	50µl* × ____ (SVF) = _____µl (Custom Volume)

### 4.B. Related Products

For a complete list of products related to this system please see the Promega product catalog at: [www.promega.com/catalog/](http://www.promega.com/catalog/)

#### 4.C. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: [www.promega.com](http://www.promega.com). E-mail: [techserv@promega.com](mailto:techserv@promega.com)

Symptoms	Causes and Comments
Clouding of lysis buffer	Heat lysis buffer at 56°C for 5-10 minutes or until buffer clears.
Clumping in binding or wash steps	Reduce sample volume. Make sure samples are mixed completely after adding Binding Buffer.
Colored elution	Make sure samples are completely lysed. Make sure all supernatant is fully aspirated during wash steps and washing is performed effectively.
Low yield	Make sure samples are completely lysed. Avoid accidental removal of MagaZorb® Reagent during aspiration steps. Allow ample time for magnetic separation. Increase starting sample amount.
$A_{260}/A_{280}$ ratio is low	Make sure samples are completely lysed. Make sure samples are completely mixed after addition of Binding Buffer. Make sure all supernatant is aspirated during wash steps and washing is performed effectively.
Blood: No color change after 56°C lysis step	Make sure both PK and Lysis Buffers are added prior to lysis incubation. Ensure incubation temperature is 56°C.
Tissue: Tissue particulates do not dissolve in lysis step.	Completely homogenize tissue at the start of processing. Make sure both PK and Lysis Buffer are added prior to lysis incubation. Ensure incubation temperature is 56°C.

(u)U.S. Pat. No. 6,855,499, European Pat. No. 1368629, Japanese Pat. No. 4399164 and other patents pending.

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.