



Promega

Technical Bulletin

Maxwell® 16 Total RNA Purification Kit

INSTRUCTIONS FOR USE OF PRODUCT AS1050.



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Maxwell[®] 16 Total RNA Purification Kit

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1. Description

The Maxwell[®] 16 Total RNA Purification Kit^(a) is used with the Maxwell[®] 16 Instrument (Cat.# AS2000) to provide an easy method for efficient, automated purification of total RNA from a variety of sample types. The Maxwell[®] 16 Instrument is supplied with preprogrammed purification procedures and is designed for use with the predispensed-reagent cartridges, maximizing simplicity and convenience. After lysate clearing, the instrument can process up to 16 samples in approximately 30 minutes. The purified total RNA can be used directly in a variety of downstream applications.

1. Description (continued)

The successful isolation of intact RNA requires four steps:

- 1) effective disruption of cells or tissue; 2) denaturation of nucleoprotein complexes; 3) inactivation of endogenous ribonucleases (RNases); and 4) removal of contaminating DNA and protein.

The most important step is the immediate inactivation of endogenous RNases, which are released from membrane-bound organelles upon cell disruption.

The Maxwell® 16 Total RNA Purification Kit combines the disruptive and protective properties of guanidine thiocyanate (GTC) to lyse samples, denature nucleoprotein complexes and inactivate ribonucleases (1). Adding β -mercaptoethanol (BME) is strongly recommended for samples containing high levels of nucleases (e.g., spleen or pancreas tissue). Genomic DNA is selectively removed from the sample lysate using the Clearing Agent. Total RNA is captured from the cleared sample lysate using MagneSil® PMPs and is further purified from contaminating salts, proteins and cellular impurities by ethanol washes. Finally, total RNA is eluted in Nuclease-Free Water.

2. Product Components and Storage Conditions

Product	Size	Cat.#
Maxwell® 16 Total RNA Purification Kit	48 preps	AS1050

For Laboratory Use. Each system contains sufficient reagents for 48 purifications. Includes:

- 48 Maxwell® 16 RNA Cartridges
- 50ml Lysis Buffer
- 44ml RNA Dilution Buffer (RDB)
- 6.5ml Clearing Agent (CAA)
- 25ml Nuclease-Free Water
- 900 μ l β -Mercaptoethanol, 97.4%
- 50 Clearing Columns
- 50 Collection Tubes
- 50 Plungers
- 50 Elution Tubes

Storage Conditions: Store β -Mercaptoethanol (BME) at 4°C. Store the remainder of the Maxwell® 16 Total RNA Purification Kit at 15–30°C. Once BME has been added to Lysis Buffer, store at 4°C for up to one month.

Safety Information: The reagent cartridges contain ethanol and guanidine thiocyanate. These should be considered flammable, harmful and irritants. β -Mercaptoethanol is toxic. Wear gloves and follow standard laboratory procedures.



The Maxwell® 16 reagent cartridges are designed to be used with potentially infectious substances. Users should wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Users should adhere to their institutional guidelines for the handling and disposal of all infectious substances when used with this system.

The Maxwell® 16 reagent cartridges contain potentially hazardous chemicals. Users should wear protective gloves or other protective means when handling the reagent cartridges. Users should follow their institutional guidelines for disposal.

Notes:

1. The RNA Dilution Buffer is colored blue so that it can be easily distinguished from the other solutions.
2. The contents of well #4 of the RNA cartridge are colored yellow to help the user distinguish Maxwell® 16 RNA Cartridges from the Maxwell® 16 Genomic DNA Cartridges.
3. Red Blood Cell Lysis Solution (CLB) is required to process white blood cells from fresh whole blood and needs to be purchased separately.

Items Available Separately

Product	Size	Cat.#
Lysis Buffer	50ml	Z3052
Red Blood Cell Lysis Solution (CLB)	200ml	Z3141

For Laboratory Use.

Note: Due to the toxicity of chemicals used in the RNA purification procedure and the prevalence of RNases, wear gloves throughout the sample and cartridge preparation procedures.

3. Processing Capacity

Table 1 describes the processing capacity of the Maxwell® 16 Total RNA Purification Kit.

Table 1. Recommended Sample Amounts.

Sample Type	Processing Capacity
Animal tissue	5–50mg
Mammalian culture cells	1 × 10 ⁵ to 5 × 10 ⁶ cells
Stabilized blood sample (blood collected into PAXgene® tubes)	1 tube ¹
White blood cell fraction from human whole blood	White blood cell fraction from 1–5ml whole blood
Plant leaf tissue	10–100mg
Aqueous phase of a phenol-chloroform procedure (e.g., TRIzol® Reagent; see Section 7.A)	Aqueous phase prepared from 5–50mg of tissue
RNA cleanup (e.g., in vitro transcription reaction; see Section 7.A)	Up to 750µl (250µg)

¹Blood stabilization tubes generally collect approximately 2ml of whole blood.

Table 2. Typical Yield of Total RNA.

Sample Type	Sample Size	Typical Yield (µg)	Typical Purity (A ₂₆₀ /A ₂₈₀)
Animal tissue (mouse liver)	10mg 50mg	40 175	2.0 2.0
Mammalian culture cells (HeLa cells)	5 × 10 ⁶ cells	up to 45 ¹	2.0
Stabilized blood sample (e.g., blood collected into PAXgene® tubes)	1 tube	up to 5 ²	1.8
White blood cell fraction from human whole blood	5ml fresh blood	up to 5 ²	1.8
Plant leaf tissue	100mg	30	2.0

¹Yield from mammalian culture cells depends on the cell type.

²Yield from whole blood will depend on the white cell count of the sample.

4. RNA Purification Procedure

Materials to be Supplied by the User

- heat block set to 65–75°C
- microcentrifuge

Specialized Materials

- rotor-stator tissue homogenizer (e.g., Tissue-Tearor™, BioSpec Products, Inc.)
- liquid nitrogen or dry ice-ethanol bath to flash freeze tissue samples
- Red Blood Cell Lysis Solution (Cat.# Z3141) for purification of white blood cells from fresh whole blood
- mortar and pestle for grinding plant samples
- trypsin and 1X PBS for adherent cell cultures

Note: Section 7.B “Creating a Ribonuclease-Free Environment” provides important suggestions for obtaining intact RNA. Please read this section before processing your samples.

4.A. Preparation of Lysis Buffer

We strongly recommend using β -Mercaptoethanol (BME) with samples likely to contain high levels of nucleases (e.g., spleen, pancreas and liver). Use 10 μ l BME per 1ml Lysis Buffer. Store any remaining Lysis Buffer + BME at 4°C up to one month.

4.B. General Information for Sample Preparation

For each sample type, information is provided on the volume of Lysis Buffer to use to prepare a particular sample lysate.



The maximum volume of lysate that you can process per prep is 500 μ l.

For example, the following table illustrates the options that are available to purify RNA from mouse liver tissue.

Table 3. Example Considerations for Determining Amount of Tissue Lysate to Process per Prep.

Mouse Liver Tissue mass	Lysis Buffer Volume	Concentration of Lysate	Volume Used per Prep	Tissue Mass per Prep
1g	6.67ml	150mg/ml	330 μ l	50mg ¹
		100mg/ml	500 μ l ²	50mg
	10ml	150mg/ml	67 μ l	10mg
		100mg/ml	100 μ l	10mg

¹Maximum tissue mass that can be processed per prep.

²Maximum volume of sample lysate that can be processed per prep.

Note: Lysate volumes greater than 250 μ l will require processing through the Clearing Column twice.

4.C. Preparation of Animal Tissue Samples

Table 4. Recommended Maximum Tissue Lysate Concentrations.

Sample Type	Maximum Sample Capacity Per Isolation ¹	Maximum Lysate Concentration (Tissue Mass per ml of Lysis Buffer)	Minimum Volume of Lysis Buffer Per 10mg of Sample
Mouse liver	50mg	150mg/ml	66µl
Mouse kidney	50mg	100mg/ml	100µl
Mouse muscle	50mg	150mg/ml	66µl
Mouse heart	50mg	150mg/ml	66µl
Mouse brain	50mg	150mg/ml	66µl
Mouse lung	50mg	150mg/ml	66µl
Mouse spleen	37.5mg	75mg/ml	133µl

¹The maximum sample capacity is recommended for optimal yield and purity of purified total RNA. Exceeding these amounts risks overloading both the DNA removal step and the RNA binding to the MagneSil[®] PMPs during purification.

1. Working as quickly as possible, homogenize tissue in an appropriate volume of Lysis Buffer (with or without BME) until no visible tissue fragments remain.
2. Incubate homogenized sample on ice for 10 minutes to ensure complete sample lysis.
3. If the tissue sample lysate is too viscous to pipet easily, add more Lysis Buffer, and rehomogenize the lysate to ensure complete lysis.

Note: Incomplete sample lysis will result in low RNA yield and purity.



Keep lysates on ice as much as possible.

4. Transfer the sample lysate (up to 500µl) to a 1.5ml microcentrifuge tube.



The maximum volume of sample lysate that can be processed is 500µl.

5. Add blue RNA Dilution Buffer to the sample lysates as directed in Table 5.

Table 5. RNA Dilution Buffer Volumes.

Lysate Volume (µl)	Volume of Blue RNA Dilution Buffer
≤50	83.5µl
51-100	167µl
101-200	334µl
201-300	500µl
301-400	668µl
401-500	835µl

- Vigorously shake or vortex the bottle of Clearing Agent to resuspend the Clearing Agent resin.

! Vigorously shake the bottle of Clearing Agent by hand or vortex for approximately 2 minutes to completely resuspend the resin. Verify that the reagent is completely resuspended by tipping the bottle upside down and ensuring that there is no Clearing Agent resin stuck to the bottom of the reagent bottle.

! Failure to completely resuspend the Clearing Agent will result not only in lower total RNA yield but also carryover of contaminating genomic DNA in the purified total RNA sample.

- Add Clearing Agent to the sample as directed in Table 6.

Table 6. Volume of Clearing Agent to Add to Sample.

Quantity of Animal Tissue	Volume of Clearing Agent
5–10mg	25µl
11–20mg	50µl
21–30mg	75µl
31–40mg	100µl
41–50mg	125µl

- Vortex sample + Clearing Agent for 30 seconds to mix thoroughly.
- Place in a 70°C heat block for 3 minutes.

Note: Ensure that the heat block is set at 70°C. (A suitable range is 65–75°C.) The heated mixtures may form clumps of precipitated debris. Heating the sample with the Clearing Agent at a lower temperature will result in incomplete clearing of genomic DNA from the sample.

- Prepare a clearing column assembly for each sample by placing one Clearing Column into a Collection Tube.
- After heating, vortex the sample + Clearing Agent again for 30 seconds.
- Incubate sample at room temperature for 5 minutes to cool the sample.



Clearing Column Assembly (Step 10).

! Insufficient heating or failure to cool the sample + Clearing Agent before transfer to the Clearing Column will result in genomic DNA contamination in the purified sample. Do **not** cool the sample on ice.

- Transfer sample + Clearing Agent to the Clearing Column.

Note: The maximum volume that the Clearing Column will hold is approximately 700µl. If you have a larger volume, centrifuge the sample through the Clearing Column in aliquots of <700µl.

- Centrifuge at 12,000 × g for 2 minutes.

4.C. Preparation of Animal Tissue Samples (continued)

- Transfer flowthrough from the Collection Tube to well #1 of the Maxwell® 16 RNA Cartridge.

Notes:

There may be a small pellet of Clearing Agent in the bottom of the Collection Tube. When you transfer the flowthrough to the RNA cartridge, be careful not to disturb this small pellet.

Pay close attention to the cartridge orientation. Well #1 is the well closest to the labeled side of the cartridge. Well #7 is the well closest to the ridged side of the cartridge.

- Repeat Steps 13–15 with any remaining sample lysate + Clearing Agent using the same Clearing Column.

Note: The maximum volume of sample flowthrough that can be transferred to well #1 of the Maxwell® 16 RNA Cartridge is 1.5ml.

- Proceed to cartridge preparation instructions (Section 4.H).

4.D. Preparation of Mammalian Cell Culture Samples

Table 7. Recommended Maximum Cultured Cell Lysate Concentrations.

Sample Type	Sample Capacity Per Isolation ¹	Maximum Lysate Concentration	Volume of Lysis Buffer
Mammalian cell culture	1×10^5 – 2×10^6 cells	2×10^7 cells/ml	100µl
	2×10^6 – 5×10^6 cells	2×10^7 cells/ml	50µl per 1×10^6 cells

¹The maximum sample capacity is recommended for optimal yield and purity of purified total RNA. Exceeding these amounts risks overloading both the DNA removal step and the RNA binding to the MagneSil® PMPs during purification.

- If working with adherent cells, trypsinize cells to detach them from the bottom of the culture plate or flask.
- Pellet cells at $300 \times g$ for 5 minutes. Decant the supernatant.
- Wash cells once with ice-cold 1X PBS, and centrifuge again to pellet cells ($300 \times g$ for 5 minutes). Remove as much supernatant as possible, then vortex the cell pellet in the remaining volume to completely resuspend the pellet.
- Add an appropriate volume of Lysis Buffer (with or without BME) to the resuspended cell pellet, and vortex to lyse cells.

- If the cell lysate is too viscous to pipet easily, add more Lysis Buffer, and vortex the lysate again to ensure complete sample lysis.

Notes:

Incomplete sample lysis will result in low RNA yield and purity.

Keep lysates on ice as much as possible.


- Transfer the sample lysate (up to 500µl) to a 1.5ml microcentrifuge tube.
-  The maximum volume of sample lysate that can be processed is 500µl.
- Add blue RNA Dilution Buffer to the sample lysate as directed in Table 8.

Table 8. RNA Dilution Buffer Volumes.

Lysate Volume	Volume of Blue RNA Dilution Buffer
51–100µl	167µl
101–200µl	334µl
201–300µl	500µl
301–400µl	668µl
401–500µl	835µl



- Vigorously shake or vortex the bottle of Clearing Agent to resuspend the Clearing Agent resin.
-  Vigorously shake the bottle of Clearing Agent by hand or vortex for approximately 2 minutes to completely resuspend the resin. Verify that the reagent is completely resuspended by tipping the bottle upside down and ensuring that there is no Clearing Agent resin stuck to the bottom of the reagent bottle.
-  Failure to completely resuspend the Clearing Agent will result not only in lower total RNA yield but also carryover of contaminating genomic DNA in the purified total RNA sample.
- Add Clearing Agent to the sample as directed in Table 9.

Table 9. Volume of Clearing Agent to Add to Sample.

Quantity of Mammalian Culture Cells	Volume of Clearing Agent
1×10^5 – 5×10^5	25µl
5.1×10^5 – 1×10^6	75µl
1.1×10^6 – 5×10^6	125µl

- Vortex sample + Clearing Agent for 30 seconds to mix thoroughly.

4.D. Preparation of Mammalian Cell Culture Samples (continued)

11. Place in a 70°C heat block for 3 minutes.

Note: Ensure that the heat block is set at 70°C. (A suitable range is 65–75°C). The heated mixtures may form clumps of precipitated debris. Heating the sample with the Clearing Agent at a lower temperature will result in incomplete clearing of genomic DNA from the sample.


12. Prepare a clearing column assembly for each sample by placing one Clearing Column into a Collection Tube.



13. After heating, vortex the sample + Clearing Agent again for 30 seconds.

14. Incubate sample at room temperature for 5 minutes to cool the sample.

Clearing Column
Assembly (Step 12).

 Insufficient heating or failure to cool the sample + Clearing Agent before transfer to the Clearing Column will result in genomic DNA contamination in the purified sample. Do **not** cool sample on ice.

15. Transfer sample + Clearing Agent to the Clearing Column.

Note: The maximum volume that the Clearing Column will hold is approximately 700µl. If you have a larger volume, centrifuge the sample through the Clearing Column in aliquots of <700µl.

16. Centrifuge at 12,000 × g for 2 minutes.

17. Transfer flowthrough from the Collection Tube to well #1 of the Maxwell® 16 RNA Cartridge.

Notes:

There may be a small pellet of Clearing Agent in the bottom of the Collection Tube. When you transfer the flowthrough to the RNA cartridge, be careful not to disturb this small pellet.

Pay close attention to the cartridge orientation. Well #1 is the well closest to the labeled side of the cartridge. Well #7 is the well closest to the ridged side of the cartridge.

18. Repeat Steps 15–17 with any remaining sample lysate + Clearing Agent using the same Clearing Column.

Note: The maximum volume of sample flowthrough that can be transferred to well #1 of the Maxwell® 16 RNA Cartridge is 1.5ml.

19. Proceed to cartridge preparation instructions (Section 4.H).

4.E. Preparation of Plant Tissue Samples

Table 10. Recommended Maximum Lysate Concentrations.

Sample Type	Maximum Sample Capacity Per Isolation ¹	Maximum Lysate Concentration (tissue mass per ml of lysis buffer)	Minimum Volume of Lysis Buffer Per 10mg of Sample
Plant tissue ²	100mg	200mg/ml	50µl

¹The maximum sample capacity is recommended for optimal yield and purity of purified total RNA. Exceeding these amounts risks overloading both the DNA removal step and the RNA binding to the MagneSil® PMPs during purification.

²The maximum lysate concentration may vary depending on plant tissue type.

1. Freeze the plant tissue in liquid nitrogen.
2. Grind the tissue into a fine powder using a mortar and pestle. Keep tissue frozen, adding liquid nitrogen as needed.
3. Add an appropriate volume of Lysis Buffer (with or without BME) to plant tissue powder, and pipet to mix.
4. Transfer the lysate to a tube, and vortex to mix.
Note: Keep lysates on ice as much as possible.
5. Transfer the sample lysate (up to 500µl) to a 1.5ml microcentrifuge tube.



The maximum volume of sample lysate that can be processed is 500µl.

6. Add blue RNA Dilution Buffer to the sample lysate as directed in Table 11.

Table 11. RNA Dilution Buffer Volumes.

Lysate Volume	Volume of Blue RNA Dilution Buffer
≤50µl	83.5µl
51-100µl	167µl
101-200µl	334µl
201-300µl	500µl
301-400µl	668µl
401-500µl	835µl

4.E. Preparation of Plant Tissue Samples (continued)

- Vigorously shake or vortex the bottle of Clearing Agent to resuspend the Clearing Agent resin.

! Vigorously shake the bottle of Clearing Agent by hand or vortex for approximately 2 minutes to completely resuspend the resin. Verify that the reagent is completely resuspended by tipping the bottle upside down and ensuring that there is no Clearing Agent resin stuck to the bottom of the reagent bottle.

! Failure to completely resuspend the Clearing Agent will result not only in lower total RNA yield but also carryover of contaminating genomic DNA in the purified total RNA sample.

- Add Clearing Agent to the sample as directed in Table 12.

Table 12. Volume of Clearing Agent to Add to Sample.

Quantity of Plant Tissue	Volume of Clearing Agent
10mg	25µl
11–20mg	50µl
21–30mg	75µl
31–40mg	100µl
41–100mg	125µl

- Vortex sample + Clearing Agent for 30 seconds to mix thoroughly.

- Place in a 70°C heat block for 3 minutes.

Note: Ensure that the heat block is set at 70°C. (A suitable range is 65–75°C). The heated mixtures may form clumps of precipitated debris. Heating the sample with the Clearing Agent at a lower temperature will result in incomplete clearing of genomic DNA from the sample.

- Prepare a clearing column assembly for each sample by placing one Clearing Column into a Collection Tube.



- After heating, vortex the sample + Clearing Agent again for 30 seconds.

- Incubate sample at room temperature for 5 minutes to cool the sample.

Clearing Column Assembly (Step 11).

Note: Insufficient heating or failure to cool the sample + Clearing Agent before transfer to the Clearing Column will result in genomic DNA contamination in the purified sample. Do **not** cool sample on ice.

- Transfer sample + Clearing Agent to the Clearing Column.

Note: The maximum volume that the Clearing Column will hold is approximately 700µl. If you have a larger volume, centrifuge the sample through the Clearing Column in aliquots of <700µl.

15. Centrifuge at $12,000 \times g$ for 2 minutes.
16. Transfer flowthrough from the Collection Tube to well #1 of the Maxwell® 16 RNA Cartridge.

Notes:

There may be a small pellet of Clearing Agent in the bottom of the Collection Tube. When you transfer the flowthrough to the RNA cartridge, be careful not to disturb this small pellet.

Pay close attention to the cartridge orientation. Well #1 is the well closest to the labeled side of the cartridge. Well #7 is the well closest to the ridged side of the cartridge.

17. Repeat Steps 14–16 with any remaining sample lysate + Clearing Agent using the same Clearing Column.

Note: The maximum volume of sample flowthrough that can be transferred to well #1 of the Maxwell® 16 RNA Cartridge is 1.5ml.

18. Proceed to cartridge preparation instructions (Section 4.H).

4.F. Preparation of Stabilized Blood Samples

Generally, a maximum of one tube of whole blood collected into stabilization solution can be processed per Maxwell® 16 Total RNA Cartridge.

1. Collect whole blood sample into an evacuated collection tube containing blood sample stabilization solution (e.g., PAXgene® tube).
2. Centrifuge the evacuated tube containing the stabilized blood solution for 10 minutes at $3,000 \times g$ to pellet the white blood cells.
3. Decant the supernatant to waste. Add 5ml of deionized water, and vortex to resuspend the cell pellet.
4. Centrifuge the tube for 10 minutes at $3,000 \times g$ to pellet the white blood cells. Decant the supernatant to waste.
5. Vortex the white blood cell pellet for 15 seconds or until the white cell pellet is completely resuspended in the remaining buffer.
6. Add 500µl of Lysis Buffer (with or without BME).
7. Vortex to mix and lyse the cell pellet.

Note: Keep lysates on ice as much as possible.

8. Transfer up to 500µl of sample lysate to a 1.5ml microcentrifuge tube.



The maximum volume of sample lysate that can be processed is 500µl.

9. Add 835µl of blue RNA Dilution Buffer to the sample lysate.

4.F. Preparation of Stabilized Blood Samples (continued)

10. Vigorously shake or vortex the bottle of Clearing Agent to resuspend the Clearing Agent resin.

ⓘ Vigorously shake the bottle of Clearing Agent by hand or vortex for approximately 2 minutes to completely resuspend the resin. Verify that the reagent is completely resuspended by tipping the bottle upside down and ensuring that there is no Clearing Agent resin stuck to the bottom of the reagent bottle.

ⓘ Failure to completely resuspend the Clearing Agent will result not only in lower total RNA yield but also carryover of contaminating genomic DNA in the purified total RNA sample.

11. Add 125µl of Clearing Agent to the sample.

12. Vortex sample + Clearing Agent for 30 seconds to mix thoroughly.

13. Place in a 70°C heat block for 3 minutes.

Note: Ensure that the heat block is set at 70°C. (A suitable range is 65–75°C). The heated mixtures may form clumps of precipitated debris. Heating the sample with the Clearing Agent at a lower temperature will result in incomplete clearing of genomic DNA from the sample.

14. Prepare a clearing column assembly for each sample by placing one Clearing Column into a Collection Tube.



15. After heating, vortex the sample + Clearing Agent again for 30 seconds.

16. Incubate sample at room temperature for 5 minutes to cool the sample.

**Clearing Column
Assembly (Step 14).**

Note: Insufficient heating or failure to cool the sample + Clearing Agent before transfer to the Clearing Column will result in genomic DNA contamination in the purified sample. Do **not** cool sample on ice.

17. Transfer sample + Clearing Agent to the Clearing Column.

Note: The maximum volume that the Clearing Column will hold is approximately 700µl. If you have a larger volume, centrifuge the sample through the Clearing Column in aliquots of <700µl.

18. Centrifuge at 12,000 × g for 2 minutes.

19. Transfer flowthrough from the Collection Tube to well #1 of the Maxwell® 16 RNA Cartridge.

Notes:

There may be a small pellet of Clearing Agent in the bottom of the Collection Tube. When you transfer the flowthrough to the RNA cartridge, be careful not to disturb this small pellet.

Pay close attention to the cartridge orientation. Well #1 is the well closest to the labeled side of the cartridge. Well #7 is the well closest to the ridged side of the cartridge.


20. Repeat Steps 17–19 with any remaining sample lysate + Clearing Agent using the same Clearing Column.

Note: The maximum volume of sample flowthrough that can be transferred to well #1 of the Maxwell® 16 RNA Cartridge is 1.5ml.

21. Proceed to cartridge preparation instructions (Section 4.H).

4.G. Preparation of Fresh White Blood Cell Samples

Generally, the white blood cells collected from 1–5ml of human whole blood sample can be processed using the Maxwell® 16 Total RNA Purification Kit. Purification of total RNA from blood cells not collected into a blood stabilization tube (e.g., PAXgene® tube) must be purified immediately after it is collected. If the blood has been collected into a stabilization tube, follow the protocol in Section 4.F. “Preparation of Stabilized Blood Samples”.

 To prepare lysate from fresh blood samples, the Red Blood Cell Lysis Solution (Cat.# Z3141) is required and needs to be purchased separately.

1. Transfer up to 5ml of fresh whole blood to a centrifuge tube.
2. Add 2 volumes of Red Blood Cell Lysis Solution (Cat.# Z3141; not included in kit), and mix by inversion.
3. Collect the blood cells by centrifugation at $400 \times g$ for 5 minutes to make a relatively clear supernatant (~30% of the volume) and a large cell pellet at the bottom of the tube.
4. Remove the supernatant to waste by pipetting from the top. Be careful not to disturb the cell pellet.
5. Repeat Steps 2–4 for a total of three times. Add the same volume of Red Blood Cell Lysis Solution each time.
6. Remove the final supernatant to waste leaving the cell pellet in a small volume of liquid at the bottom of the tube.
7. Resuspend the final cell pellet by vortexing for 15 seconds or until the cell pellet is completely resuspended.

4.G. Preparation of Fresh White Blood Cell Samples (continued)

8. Add 500µl of Lysis Buffer (with or without BME), and vortex to mix and lyse.

Note: Keep lysates on ice as much as possible.

9. Transfer up to 500µl of sample lysate to a 2.0ml microcentrifuge tube.

! The maximum volume of sample lysate that can be processed is 500µl.

10. Add 835µl of blue RNA Dilution Buffer to the sample lysate.

11. Vigorously shake or vortex the bottle of Clearing Agent to resuspend the Clearing Agent resin.

! Vigorously shake the bottle of Clearing Agent by hand or vortex for approximately 2 minutes to completely resuspend the resin. Verify that the reagent is completely resuspended by tipping the bottle upside down and ensuring that there is no Clearing Agent resin stuck to the bottom of the reagent bottle.

! Failure to completely resuspend the Clearing Agent will result not only in lower total RNA yield but also carryover of contaminating genomic DNA in the purified total RNA sample.

12. Add Clearing Agent to the sample as directed in Table 13.

Table 13. Volume of Clearing Agent to Add to Sample.

Quantity of Fresh Blood Sample	Volume of Clearing Agent
1ml	62.5µl
2ml	125µl
3ml	187.5µl
4ml	250µl
5ml	312.5µl

13. Vortex sample + Clearing Agent for 30 seconds to mix thoroughly.

14. Place in a 70°C heat block for 3 minutes.

Note: Ensure that the heat block is set at 70°C. (A suitable range is 65–75°C). The heated mixtures may form clumps of precipitated debris. Heating the sample with the Clearing Agent at a lower temperature will result in incomplete clearing of genomic DNA from the sample.

15. Prepare a clearing column assembly for each sample by placing one Clearing Column into a Collection Tube.



**Clearing Column
Assembly (Step 15).**

16. After heating, vortex the sample + Clearing Agent again for 30 seconds.

17. Incubate sample at room temperature for 5 minutes to cool the sample.

Note: Insufficient heating or failure to cool the sample + Clearing Agent before transfer to the Clearing Column will result in genomic DNA contamination in the purified sample. Do **not** cool sample on ice.

18. Transfer sample + Clearing Agent to the Clearing Column.

Note: The maximum volume that the Clearing Column will hold is approximately 700µl. If you have a larger volume, centrifuge the sample through the Clearing Column in aliquots of <700µl.

19. Centrifuge at 12,000 × g for 2 minutes.

20. Transfer flowthrough from the Collection Tube to well #1 of the Maxwell® 16 RNA Cartridge.

Notes:

There may be a small pellet of Clearing Agent in the bottom of the Collection Tube. When you transfer the flowthrough to the RNA cartridge, be careful not to disturb this small pellet.

Pay close attention to the cartridge orientation. Well #1 is the well closest to the labeled side of the cartridge. Well #7 is the well closest to the ridged side of the cartridge.

21. Repeat Steps 18–20 with any remaining sample lysate + Clearing Agent using the same Clearing Column.

Note: The maximum volume of sample flowthrough that can be transferred to well #1 of the Maxwell® 16 RNA Cartridge is 1.5ml.

22. Proceed to cartridge preparation instructions (Section 4.H).

4.H. Cartridge Preparation

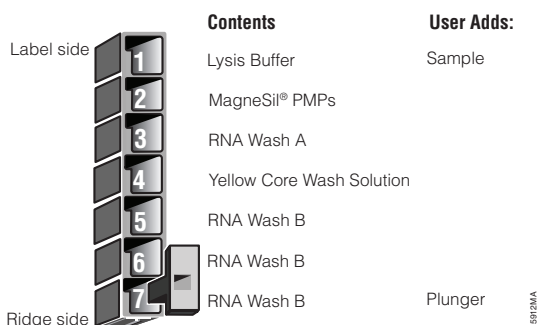
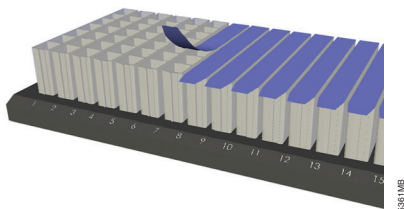


Figure 1. Overview of the Maxwell® 16 Total RNA Purification cartridge.

4.H. Cartridge Preparation (continued)

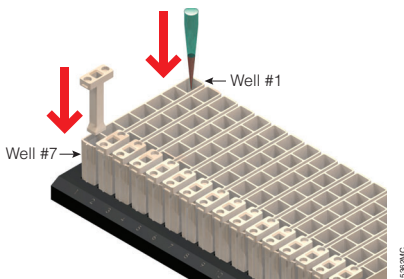


1. Place the number of cartridges to be used into the cartridge preparation rack. Place each cartridge into the holder with the ridged side of the cartridge facing toward the numbered side of the rack. Remove the seal from each cartridge. Cartridge label (well #1) will be facing away.

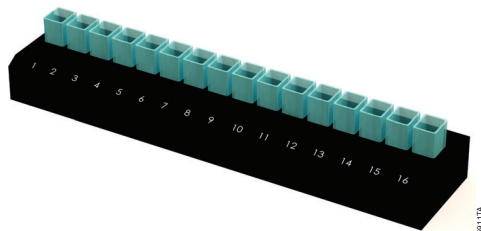


The Maxwell® 16 reagent cartridges are designed to be used with potentially infectious substances. Users should wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Users should adhere to their institutional guidelines for the handling and disposal of all infectious substances when used with this system.

The Maxwell® 16 reagent cartridges contain potentially hazardous chemicals. Users should wear protective gloves or other protective means when handling the reagent cartridges. Users should follow their institutional guidelines for disposal.



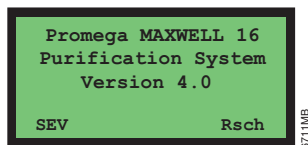
2. Add your flowthrough from the Clearing Column to well #1. Well #1 is the well closest to the labeled side of the cartridge.
3. Place one plunger into cartridge well #7. Well #7 is the closest to the ridged side of the cartridge.



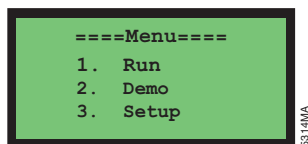
4. Prepare elution tubes by adding 300µl of Nuclease-Free Water to one blue Elution Tube per purification.

4.I. RNA Purification on the Maxwell® 16 Instrument

To use the Maxwell® 16 Total RNA Purification Kit (Cat.# AS1050) , the Maxwell® 16 Instrument must be configured with Maxwell® 16 SEV Hardware Kit (Cat.# AS1200). Reconfiguring the instrument is simple and easy. Please refer to the *Maxwell® 16 Instrument Technical Manual* for directions.



1. Verify that the instrument mode is set to Research. Do this by closing the door and turning the Maxwell® 16 Instrument off, then on again. The instrument will power up and display the firmware version number , operational mode and hardware configuration. Verify that “Rsch” and “SEV” are displayed as shown. If it is not, refer to the instrument Technical Manual for instructions on how to reset the instrument mode.

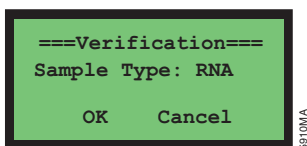


2. Use the Scroll Up or Scroll Down button to move the cursor to “Run” to perform a purification run. Press “Run/Stop” to select.

Note: “Demo” is an abbreviated purification run for demonstration purposes. “Setup” is used only to change the mode of the instrument.

4.I. RNA Purification on the Maxwell® 16 Instrument (continued)

3. Use the Scroll Up or Scroll Down button to move the cursor to "RNA". Press "Run/Stop" to select.



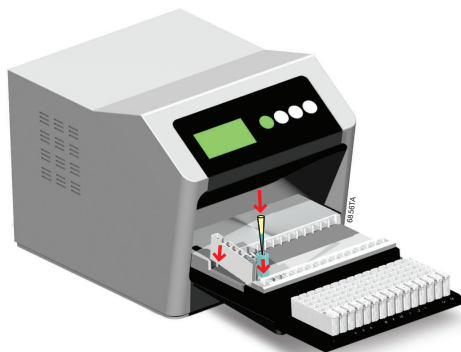
4. Verify that you have selected the correct protocol. Use the Scroll Up or Scroll Down button to move the cursor to "OK". Press the "Run/Stop" button to start the purification run. Select "Cancel" if the information displayed is not correct.



5. Open the door when prompted to do so on the LCD display.
6. Press the "Run/Stop" button to extend the platform out of the instrument for easy insertion of the cartridges.



Warning: Pinch point hazard.



7. Transfer cartridges containing samples and plungers from the cartridge preparation rack onto the Maxwell® 16 Instrument platform.

Notes:

Ensure that the cartridges are placed into the Maxwell® 16 Instrument with the ridged side of the cartridge closest to the door. The cartridges will only fit into the instrument in this orientation.

It is easiest to insert the ridged side of the cartridge first, then press down on the back of the cartridge to “click” it into place.

If you are processing fewer than 16 samples, center the reagent cartridges on the platform, spacing them evenly outward from the center.

8. Ensure the blue Elution Tubes containing 300µl of Nuclease-Free Water are placed in the heated elution positions on the instrument platform.



9. Press the “Run/Stop” button. The platform will retract. Close the door.




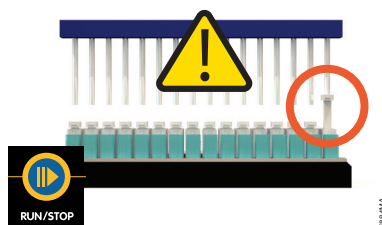
Warning: Pinch point hazard.

4.I. RNA Purification on the Maxwell® 16 Instrument (continued)

10. The Maxwell® 16 Instrument will begin the purification run. The LCD screen will display the steps performed and the approximate time remaining in the run.

Note: Pressing the “Run/Stop” button or opening the door will pause the run. Close the door if open, and select whether to “continue” or “terminate”.

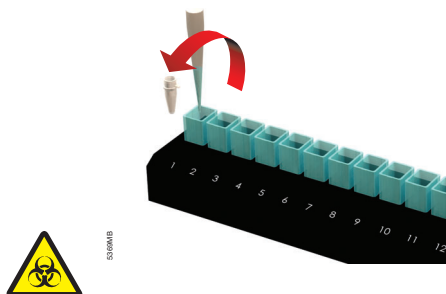
 If you choose to terminate the program before completion, the instrument will wash the particles off the plungers, and **you will lose your sample**.



11. When the purification is complete, the LCD screen will display a message that the method has ended.

Upon method completion, open the instrument door. Check to make sure that all plungers have been removed from the magnetic rod assembly. If the plungers have not been removed, gently push them down by hand to remove them from the magnetic rod assembly.

12. Press the “Run/Stop” button to extend the platform out from the instrument.



13. Remove the Elution Tubes from the platform, and place them into the Magnetic Elution Tube Rack, keeping the tubes in the same order. Transfer the eluted samples into storage tubes by pipetting.

Note: To avoid particle transfer, use a pipette tip to aspirate samples away from the captured particles on the side of the blue Elution Tube.



14. Remove cartridges and plungers from the instrument platform, and discard them. **Do not** reuse reagent cartridges, plungers or Elution Tubes.

5. Determining RNA Yield and Quality

The most common method to determine the yield and purity of RNA is spectrophotometry. The yield of the total RNA is determined by measuring the sample absorbance at 260nm. According to Beer's Law, an absorbance unit ($A_{260} = 1.0$) equals $\sim 40\mu\text{g}/\text{ml}$ of single-stranded RNA in a 1cm path length cuvette. Table 2 shows representative yields of total RNA isolated using the Maxwell® 16 Total RNA Purification Kit. The total RNA yield will vary depending on the type of starting material.

Note: If you are using a 1cm square cuvette, the cuvette should be UV transparent (e.g., quartz). Disposable cuvettes may not be UV transparent.

The A_{260}/A_{280} ratio is an indicator of protein contamination. Pure RNA will exhibit an A_{260}/A_{280} ratio greater than 1.8. If the sample is contaminated with protein, the ratio will be lower due to an increased absorbance at 280nm.

The integrity of purified RNA may be determined by denaturing agarose gel electrophoresis. The ratio of 28S to 18S ribosomal RNA should be approximately 2:1 by ethidium bromide staining. This indicates that no substantial degradation of RNA has occurred. For RNA samples that have been degraded, this ratio will be reversed since the 28S ribosomal RNA is characteristically degraded into 18S-like species.

6. Troubleshooting

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

Symptoms	Causes and Comments
Lysate foamed during homogenization	Some homogenizers generate foam when samples are homogenized. Allow the foam to dissipate before pipetting. Homogenize for shorter periods of time until visible particles and tissue fragments are eliminated.
Lysate was too viscous to pipet	The lysate was too concentrated or became viscous while sitting on ice. Reduce the lysate viscosity by increasing the amount of Lysis Buffer 1.5- to 2-fold, and briefly rehomogenize the sample. The maximum volume that can be processed is 500µl of initial sample lysate. Note: Rehomogenization can result in lower yields due to overheating. Therefore, only rehomogenize when necessary and on ice if possible.
Clearing Column clogged	<p>Homogenization was incomplete. Incomplete homogenization of samples results in clumps of debris and particulates that may clog the Clearing Columns.</p> <p>Lysate was too concentrated. If the lysate was difficult to pipet, the lysate was too concentrated and exceeded the clearing capacity. Reduce the lysate viscosity by increasing the amount of Lysis Buffer. (See symptom "Lysate was too viscous to pipet".)</p> <p>Incomplete mixing. The Clearing Agent settles quickly in the lysate. Vortex the lysate mixture immediately before adding it to the Clearing Column.</p> <p>Too much sample was processed. Do not exceed the sample amounts recommended in Table 1. For sample types that are not listed, choose the most similar sample type as a guideline. Reduce lysate concentration twofold for samples that clog the columns.</p>

Symptoms

Low RNA yield, RNA degradation or poor reproducibility between samples

Causes and Comments

Homogenization was incomplete. Incomplete homogenization of samples resulted in loss of RNA within the particulate and clumps of debris. Incubate the lysate on ice for 10 minutes to ensure complete lysis.

RNA was degraded during sample preparation. It is essential to work quickly during sample preparation. Maintain the sample lysate at 4°C during preparation. We strongly recommend adding BME to Lysis Buffer for samples with high nuclease content.

Samples were not properly prepared or stored. To halt RNA degradation within samples, flash freeze or immediately homogenize sample with Lysis Buffer. Delays in sample collection may result in RNA degradation and lower yields. Freeze samples immediately in liquid nitrogen, and store at -70°C if they cannot be immediately processed. Lysates should be stored at -70°C and thawed on ice.

Frozen samples thawed before homogenization will result in the release of RNases and degradation of RNA. Keep samples frozen on dry ice until they can be homogenized in Lysis Buffer.

Frozen lysate was heated to thaw. Thaw frozen lysates on ice. To prevent RNA degradation, crude lysates should never be heated above 22–25°C.

Sample contained a low abundance of RNA. The amount of RNA present in a sample depends on the metabolic state, stage of growth, type of sample and growth conditions. Sample types vary in the amount of total RNA.

Too much Clearing Agent was used. Reduce the volume of Clearing Agent, or titrate the volume of Clearing Agent to determine the optimal amount to use.

Inconsistent yield and purity from stabilized blood cells or white blood cells from fresh blood. Purified RNA yield and quality depends on cell number, cell state, amount of time elapsed from blood draw, sample storage conditions, etc.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low RNA yield, RNA degradation or poor reproducibility between samples (continued)	<p>Samples were not heated properly after adding the Clearing Agent. The heating step is critical for high RNA yields and reproducibility. Incomplete heating will result in decreased yields and decreased reproducibility.</p> <hr/> <p>The sample was not cooled properly before adding it to the Clearing Column. Be sure to cool the sample at room temperature for 5 minutes before adding it to the Clearing Column.</p> <hr/> <p>RNase was introduced by handling. Use sterile, disposable plasticware or baked glassware when handling RNA. Wear clean gloves at all times. Mishandling of kit plastics and introduction of RNases during or after purification will degrade the RNA. See Section 7.B "Creating a Ribonuclease-Free Environment."</p>
DNA contamination seen when performing RT-PCR or PCR	<p>Too much sample was processed. When the suggested sample amounts are used, most purified RNA samples do not show any DNA contamination in RT-PCR. However, dense tissues or cultures may contain too much DNA to eliminate. Reduce the starting sample amount by twofold.</p> <hr/> <p>Sample had an excessive amount of genomic DNA. Reduce the starting sample amount by twofold, or increase the volume of Clearing Agent.</p> <hr/> <p>Heating step was insufficient. Heating the sample lysate with the Clearing Agent is essential to eliminate DNA contamination. If the samples are not adequately heated, genomic DNA contamination may occur.</p> <hr/> <p>Sample was not completely cooled before transfer to Clearing Column. Be sure to cool the sample before adding it to the Clearing Column.</p> <hr/> <p>Possible cross-contamination during preparation of RT-PCR and PCR. Use aerosol-resistant pipette tips to set up reactions. Analyze samples in a separate location.</p>

<u>Symptoms</u>	<u>Causes and Comments</u>
DNA contamination seen when performing RT-PCR or PCR (continued)	<p>Too much sample was used in the RT-PCR. Reduce the total RNA input to 50-100ng in RT-PCR. Generally, a rare message can be detected in 50ng of total RNA by RT-PCR.</p> <hr/> <p>Clearing Agent was not used or not mixed thoroughly before use. The Clearing Agent is required to remove genomic DNA. Thorough mixing before dispensing ensures that the proper amount of Clearing Agent is used. To resuspend the Clearing Agent, shake by hand or vortex vigorously for at least 2 minutes. Turn the reagent bottle upside down and verify that no Clearing Agent resin is stuck to the bottom of the reagent bottle. If there is, repeat vigorous shaking or vortexing until no Clearing Agent resin is visible on the bottom of the reagent bottle.</p> <hr/> <p>Samples were not mixed thoroughly. The lysate should be vortexed with the RNA Dilution Buffer as well as the Clearing Agent.</p>
Purified total RNA appears cloudy	<p>Total RNA purified from liver may contain glycogen. When stored at 4°C or frozen, the glycogen may form a precipitate and the sample may appear cloudy. Warm the sample to 22-25°C to redissolve the glycogen. Glycogen does not interfere in reactions that use nucleic acids as a substrate.</p>

7. Appendix

7.A. RNA Cleanup Application

The procedure below applies to RNA samples that require cleanup from an aqueous solution (e.g., in vitro transcription reactions or the aqueous phase from a phenol-chloroform extraction). The maximum volume of sample that you can process per Maxwell® 16 RNA Cartridge is 500µl.

1. Optional DNase treatment to remove DNA: Add 1 unit of RQ1 RNase-Free DNase (Cat.# M6101) to the sample. Incubate at 37°C for 15 minutes. (For an in vitro transcription reaction, add 1 unit of RQ1 RNase-Free DNase per 1µg of plasmid used in the reaction.)
2. Transfer the reaction volume to well #1 of the Maxwell® 16 RNA Cartridge.
3. Proceed to “Cartridge Preparation” and “RNA Purification on the Maxwell® 16 Instrument” (Sections 4.H and 4.I).

7.B. Creating a Ribonuclease-Free Environment

Ribonucleases are very difficult to inactivate. Take care to avoid inadvertently introducing RNase activity into your RNA during or after the isolation procedure. This is especially important if the starting material is difficult to obtain or is irreplaceable. The following notes may help you prevent accidental RNase contamination of your sample.

1. Two of the most common sources of RNase contamination are the user’s hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use sterile technique when handling reagents and plastics supplied with the system. Wear gloves at all times. Change gloves whenever ribonucleases may have been contacted.
2. Whenever possible, sterile disposable plasticware should be used for handling RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat nondisposable glassware and plasticware before use to ensure that it is RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA followed by RNase-free water. Commercially available RNase removal products may also be used, following the manufacturer’s instructions.

Note: Electrophoresis chambers may be contaminated with ribonucleases, particularly RNase A, from analysis of DNA samples. Whenever possible, set aside a new or decontaminated apparatus for RNA analysis only.

4. Treat solutions not supplied by Promega by adding diethyl pyrocarbonate (DEPC) to 0.1% in a fume hood. Incubate overnight at room temperature in the hood. Autoclave for 30 minutes to remove any trace of DEPC.

Caution: DEPC is a suspected carcinogen and should only be used in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

7.C. Downstream Applications

Total RNA purified with the Maxwell® 16 Total RNA Purification Kit is suitable for molecular biology applications such as RT-PCR, real-time qRT-PCR, microarrays and Northern blot analysis. For more information on downstream applications, see the *Promega Protocols and Applications Guide (2)* and *RNA Applications Guide (3)*.

Note: For all downstream applications, it is essential that you continue to protect your RNA samples from RNases. Continue to wear clean gloves, and use solutions and centrifuge tubes that are RNase-free.

7.D. Composition of Buffers and Solutions

1X PBS

137mM	NaCl
2.7mM	KCl
4.3mM	Na ₂ HPO ₄
1.47mM	KH ₂ PO ₄

The final pH should be 7.1.

7.E. References

1. Chirgwin, J.M. *et al.* (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294–9.
2. *Protocols and Applications Guide*, Online Edition (2004) Promega Corporation.
3. *RNA Applications Guide* (2003) Promega Corporation.

7.F. Related Products

Product	Size	Cat.#
Maxwell® 16 Instrument*	1 each	AS2000
Maxwell® 16 Blood DNA Purification Kit*	48 preps	AS1010
Maxwell® 16 Cell DNA Purification Kit*	48 preps	AS1020
Maxwell® 16 Tissue DNA Purification Kit*	48 preps	AS1030
Maxwell® 16 Polyhistidine Protein Purification Kit	48 preps	AS1060
Maxwell® 16 Tissue LEV Total RNA Purification Kit*	48 preps	AS1220
Maxwell® 16 Cell LEV Total RNA Purification Kit*	48 preps	AS1225
Maxwell® 16 SEV Hardware Kit	1 each	AS1200
Maxwell® 16 LEV Hardware Kit	1 each	AS1250

*For Laboratory Use.

^(*)U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756, Japanese Pat. No. 3253638, Mexican Pat. No. 209436 and other patents pending.

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