

MasterPure™ RNA Purification Kit

Cat. No. MCR85102

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1. Introduction

The MasterPure™ RNA Purification Kit provides all of the reagents necessary to recover RNA from a wide variety of biological sources. This kit uses a rapid desalting process¹ to remove contaminating macromolecules, avoiding toxic organic solvents. The purified RNA can be used subsequently in many applications including hybridization, RNase protection, and RT-PCR. We offer several products for PCR that incorporate the MasterAmp™ PCR Enhancement Technology⁺, which substantially improves product yield and decreases nonspecific product formation.

2. Product Specifications

Storage: Store the RNase-Free DNase I, Proteinase K, and RiboGuard™ RNase Inhibitor at –20°C in a freezer without a defrost cycle. Store the remainder of the kit at room temperature.

Storage Buffers: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, and 10 mM CaCl₂; Proteinase K is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 10 mM CaCl₂, 0.1% Triton® X-100, and 1 mM dithiothreitol.

Quality Control: The MasterPure RNA Purification Kit is function-tested by purifying RNA from *E. coli*. RNA quality and yield are assayed by agarose gel electrophoresis, spectrophotometry, fluorimetry, and use as a template for RT-PCR.

3. Kit Contents

Desc.	Concentration	Quantity
The MasterPure RNA Purification Kit contains enough reagents to perform 100 RNA purifications.		
Red Cell Lysis Solution		120 ml
Tissue and Cell Lysis Solution		60 ml
2XT and C Lysis Solution		50 ml
MPC Protein Precipitation Reagent		50 ml
RNase-Free DNase I	@ 1 U/μl	500 μl
Proteinase K	@ 50 mg/ml	200 μl
RiboGuard™ RNase Inhibitor	@ 40 U/μl	100 μl
1X DNase Buffer		20 ml
(33 mM Tris-HCl [pH 7.8], 66 mM potassium acetate, 10 mM magnesium acetate, and 0.5 mM dithiothreitol)		
TE Buffer		7 ml
(10 mM Tris-HCl [pH 7.5], 1 mM EDTA)		

All MasterPure RNA Purification Kit components are also available separately.

4. Related Products

The following products are also available:

- MasterPure™ Complete DNA and RNA Purification Kits
- MasterPure™ DNA Purification Kit
- MasterPure™ Yeast DNA Purification Kit
- MasterPure™ Yeast RNA Purification Kit
- MasterPure™ Plant RNA Purification Kit
- BuccalAmp™ DNA Extraction Kits
- MasterAmp™ High Fidelity RT-PCR Kits
- MasterAmp™ RT-PCR Kits for High Sensitivity
- MasterAmp™ PCR Optimization Kits
- MasterAmp™ *Taq*, *Tth*, *Tfl*, and AmpliTherm™ DNA Polymerases
- FailSafe™ PCR System

5. General Considerations

1. **Tissue Sources:** We have used the kit to isolate RNA from a variety of sources including: bovine liver, human HL-60 tissue culture cells, human whole blood and plasma, saliva, corn and geranium leaf, yeast, *E. coli*, and lambda phage. Tissues other than those mentioned here are likely to be compatible with the kit with some optimization.
2. **Isolation of RNA from Paraffin-Embedded Tissue:** RNA isolated from preserved, paraffin-embedded tissues is generally of poor quality. The degree of degradation of these samples limits analysis mainly to techniques involving amplification. To obtain RNA from embedded tissues that is amenable to RT-PCR, we recommend preserving the tissues in either acetone, 95% ethanol, or 95% buffered formalin, with fixation times of less than 24 hours. Choose primers such that the resultant amplification products are less than or equal to 300 bp in length.
3. **Sample Size:** You can purify nucleic acid from samples of various sizes by proportionally adjusting the amount of reagents to the amount of starting material. With larger samples, centrifugation conditions (time and speed) may also need to be adjusted.
4. **Proteinase K Treatment:** We recommend including the Proteinase K treatment to increase the efficiency of lysis, though for some samples this treatment is unnecessary (e.g., blood). If minimizing the time of purification is desirable, you may determine if Proteinase K treatment is required.
5. **Nuclease Treatment:** The removal of DNA from RNA preparations with RNase-Free DNase I is unnecessary for many applications. This step may be eliminated from the protocol depending upon the intended use of the RNA. If the removal of contaminating nucleic acid is necessary, we recommend performing these steps as outlined in the protocol. Note, however, for some samples, adjustments in nuclease concentration or time of incubation may improve the quality of the purified nucleic acid.

6. RNA Purification Protocols

The following protocol is provided for the purification of RNA from several biological sources (see General Considerations above). Lyse the fluid or tissue as outlined in Part A, and then proceed with the remainder of the protocol as outlined in Part B. If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined in Part C. Use appropriate techniques to minimize degradation by exogenous ribonucleases. Additional purification protocols begin on page 6.

A. Lysis of Fluid or Tissue Samples

Thoroughly mix the various Lysis Solutions to ensure uniform composition before dispensing.

Fluid Samples (e.g., saliva, semen)

1. Collect samples and either process immediately or freeze at -70°C .
2. Dilute $1\ \mu\text{l}$ of $50\ \mu\text{g}/\mu\text{l}$ Proteinase K into $150\ \mu\text{l}$ of 2X T and C Lysis Solution for each sample.
3. Transfer $150\ \mu\text{l}$ of the fluid sample to a microcentrifuge tube and, $150\ \mu\text{l}$ of 2X T and C Lysis Solution containing the Proteinase K, and mix thoroughly.
4. Incubate at 65°C for 15 minutes; vortex every 5 minutes.
5. Place the samples on ice for 3-5 minutes and then proceed with total nucleic acid precipitation in Part B.

Cell Samples (e.g., mammalian cell culture, buccal cells, *E. coli*)

1. Dilute $1\ \mu\text{l}$ of $50\ \mu\text{g}/\mu\text{l}$ Proteinase K into $300\ \mu\text{l}$ of Tissue and Cell Lysis Solution for each sample.
2. Pellet cells by centrifugation ($0.5\text{-}1 \times 10^6$ mammalian cells; $0.1\text{-}0.5\ \text{ml}$ of an overnight culture of *E. coli*) and discard the supernatant, leaving approximately $25\ \mu\text{l}$ of liquid.
3. Vortex for 10 seconds to resuspend the cell pellet.
4. Add $300\ \mu\text{l}$ of Tissue and Cell Lysis Solution containing the Proteinase K and mix thoroughly.
5. Incubate at 65°C for 15 minutes; vortex every 5 minutes.
6. Place the samples on ice for 3-5 minutes and then proceed with total nucleic acid precipitation in Part B.

Tissue Samples (e.g., plant or animal tissues)

1. Collect 1-5 mg of tissue and either process immediately or freeze the samples at -70°C .
2. Dilute $1\ \mu\text{l}$ of $50\ \mu\text{g}/\mu\text{l}$ Proteinase K into $300\ \mu\text{l}$ of Tissue and Cell Lysis Solution for each sample.
3. Homogenize fresh tissue or grind frozen tissues in liquid nitrogen and transfer to a microcentrifuge tube.
4. Add $300\ \mu\text{l}$ of Tissue and Cell Lysis Solution containing the Proteinase K and mix thoroughly.

5. Incubate at 65°C for 15 minutes; vortex every 5 minutes.
6. Place the samples on ice for 3-5 minutes and then proceed with total nucleic acid precipitation in Part B.

Whole-Blood Samples (with RBC lysis)

1. Draw 5 ml of blood into an EDTA Vacutainer® tube. Transfer 200 µl of whole blood into a microcentrifuge tube.
Note: For processing larger volumes of whole blood, the volumes of MasterPure reagents can be scaled up. See Appendix A.
2. Add 600 µl of Red Cell Lysis Solution. Invert three times to mix and then flick the bottom of the tube to suspend any remaining material.
3. Incubate at room temperature for 5 minutes and then vortex briefly. Continue incubating at room temperature for an additional 5 minutes followed again by brief vortexing.
4. Pellet the white blood cells by centrifugation for 25 seconds in a microcentrifuge.
5. Remove most of the supernatant, leaving approximately 25 µl of liquid. Vortex to suspend the pellet.
6. Resuspend the white blood cells in 300 µl of Tissue and Cell Lysis Solution by pipetting the cells several times.
7. Place the samples on ice for 3-5 minutes and then proceed with total nucleic acid precipitation in Part B.

Formalin-Fixed, Paraffin-Embedded (FFPE) Tissues (see General Considerations)

1. Remove a section of tissue using a clean microtome blade; if possible, trim excess paraffin.
2. Place 2-30 mg of 10- to 35-µm thick paraffin sections into an appropriate tube. If using a larger amount of tissue, adjust the reagent volumes accordingly.
3. Dilute 2 µl of Proteinase K into 300 µl of Tissue and Cell Lysis Solution for each sample, and mix.
4. Add 300 µl of Tissue and Cell Lysis Solution containing the Proteinase K to the sample and mix.
5. Incubate at 65°C for 30 minutes; followed by a brief (10 seconds) vortex mix.
6. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation in Part B (below).

B. Precipitation of Total Nucleic Acids (for all biological samples)

1. Add 175 μ l of MPC Protein Precipitation Reagent to 300 μ l of lysed sample and vortex vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 μ l of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
5. Pellet the total nucleic acids by centrifugation at 4°C for 10 minutes in a microcentrifuge.
6. Carefully pour off the isopropanol without dislodging the total nucleic acid pellet. If removal of contaminating DNA from the RNA is required, proceed with Part C (below). Otherwise, complete the remainder of Part B.
7. Rinse twice with 70% ethanol, being careful to not dislodge the total nucleic acid pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet.
8. Resuspend the total nucleic acids in 35 μ l of TE Buffer.

C. Removal of Contaminating DNA from Total Nucleic Acid Preparations

(for all biological samples)

1. Remove all of the residual isopropanol with a pipet.
2. Prepare 200 μ l of DNase I solution for each sample by diluting 5 μ l of RNase-Free DNase I up to 200 μ l with 1X DNase Buffer.
3. Completely resuspend the total nucleic acid pellet in 200 μ l of DNase I solution.
4. Incubate at 37°C for 10 minutes.
Note: *Additional incubation (up to 30 minutes) may be necessary to remove all contaminating DNA.*
5. Add 200 μ l of 2X T and C Lysis Solution; vortex for 5 seconds.
6. Add 200 μ l of MPC Protein Precipitation Reagent; vortex 10 seconds; place on ice for 3-5 minutes.
7. Pellet the debris by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
8. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
9. Add 500 μ l of isopropanol to the supernatant. Invert the tube 30-40 times.
10. Pellet the purified RNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.
11. Carefully pour off the isopropanol without dislodging the RNA pellet.
12. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet.
13. Resuspend the RNA in 10-35 μ l of TE Buffer.
14. Add 1 μ l of RiboGuard™ RNase Inhibitor (optional).

7. Additional Purification Protocols

The following protocol is provided for the purification of RNA from plasma. If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined on page 8. Use appropriate techniques to minimize degradation by exogenous ribonucleases.

D. Lysis of Plasma

Thoroughly mix the Tissue and Cell Lysis Solution to ensure uniform composition before dispensing.

1. Collect plasma samples. Transfer 50 μ l of plasma into a microcentrifuge tube.
2. Dilute 1 μ l of 50 μ g/ μ l Proteinase K into 400 μ l of Tissue and Cell Lysis Solution for each sample.
3. Add 400 μ l of Tissue and Cell Lysis Solution containing the Proteinase K and mix thoroughly.
4. Incubate at 65°C for 15 minutes; vortex every 5 minutes.
5. Proceed with total nucleic acid precipitation in Part E (below).

E. Precipitation of Total Nucleic Acids (from plasma lysis)

1. Place the samples on ice for 5 minutes.
2. Add 250 μ l of MPC Protein Precipitation Reagent and vortex mix vigorously for 10 seconds.
3. Pellet the debris by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
4. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
5. Add 600 μ l of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
6. Pellet the RNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.
7. Carefully pour off the isopropanol without dislodging the RNA pellet. If removal of contaminating DNA is required, proceed with Part J (page 8). Otherwise, complete the remainder of Part E.
8. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet.
9. Resuspend the RNA in 35 μ l of TE Buffer.

The following protocol is provided for the purification of RNA from whole blood without the initial lysis of the red blood cells. If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined in Part J. Use appropriate technique to minimize degradation by exogenous ribonucleases.

F. Lysis of Whole Blood (without RBC lysis)

Thoroughly mix the Tissue and Cell Lysis Solution to ensure uniform composition before dispensing.

1. Collect whole-blood samples. Transfer 12.5 µl of blood into a microcentrifuge tube.
2. Dilute 2 µl of 50 µg/µl Proteinase K into 400 µl of Tissue and Cell Lysis Solution for each sample.
3. Add 400 µl of Tissue and Cell Lysis Solution containing the Proteinase K and mix thoroughly.
4. Incubate at 65°C for 15 minutes; vortex every 5 minutes.
5. Proceed with total nucleic acid precipitation in Part G.

G. Precipitation of Total Nucleic Acids (from whole blood lysis)

1. Place the samples on ice for 5 minutes.
2. Add 225 µl of MPC Protein Precipitation Reagent and vortex vigorously for 10 seconds.
3. Pellet the debris by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
4. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
5. Add 600 µl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
6. Pellet the nucleic acid by centrifugation at 4°C for 10 minutes in a microcentrifuge.
7. Carefully pour off the isopropanol without dislodging the pellet. If removal of DNA from RNA preparations is required, proceed with the protocol in Part J (page 8). Otherwise, complete the remainder of Part G.
8. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet.
9. Resuspend the total nucleic acids in 35 µl of TE Buffer.

The following protocol is provided for the purification of RNA from the buffy coat of blood. If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined in Part J. Use appropriate techniques to minimize degradation by exogenous ribonucleases.

H. Lysis of Buffy Coat

Thoroughly mix the various Lysis Solutions to ensure uniform composition before dispensing.

1. Draw 5 ml of blood into an EDTA Vacutainer tube. Separate fractions by centrifugation at 1,000 x g for 15 minutes and carefully transfer 600 µl of buffy coat (the white interface between the plasma and the red blood cells) to a microcentrifuge tube. Transfer of some red blood cells is not detrimental to the purification of nucleic acids from buffy coat. Vortex the buffy coat sample and transfer 300 µl of the sample to another microcentrifuge tube; process the two tubes in parallel.
2. Add 1.2 ml of Red Cell Lysis Solution to each tube, invert three times to mix, and flick the bottom of the tubes to suspend any remaining material.
3. Incubate at room temperature for 5 minutes and then vortex briefly. Continue incubating at room temperature for an additional 5 minutes, followed again by brief vortexing.
4. Pellet the white blood cells by centrifugation for 25 seconds in a microcentrifuge.
5. Remove most of the supernatant, leaving approximately 25 µl of liquid. Vortex to suspend the pellets.
6. Resuspend the white blood cells in 600 µl of Tissue and Cell Lysis Solution by pipetting the cells several times.
7. Place the samples on ice for 3-5 minutes and then proceed with total nucleic acid precipitation in Part I (below).

I. Precipitation of Total Nucleic Acids (from buffy coat)

1. Add 300 µl of MPC Protein Precipitation Reagent and vortex vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 750 µl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
5. Pellet the nucleic acid by centrifugation at 4°C for 10 minutes in a microcentrifuge.
6. Carefully pour off the isopropanol without dislodging the pellet. If removal of DNA from RNA preparations is required, proceed with the protocol in Part J. Otherwise, complete the remainder of Part I.
7. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet.
8. Resuspend the total nucleic acids in 35 µl of TE Buffer.

J. Removal of Contaminating DNA from RNA Preparations

1. Remove all of the residual isopropanol with a pipet.
2. Prepare 200 μ l of DNase I solution for each sample by diluting 10 μ l of RNase-Free DNase I up to 200 μ l with 1X DNase Buffer.
3. Completely resuspend the total nucleic acid pellet in 200 μ l of DNase I solution.
4. Incubate at 37°C for 30 minutes.
5. Add 200 μ l of 2XT and C Lysis Solution; vortex for 5 seconds.
6. Add 200 μ l of MPC Protein Precipitation Reagent; vortex 10 seconds; place on ice 3-5 minutes.
7. Pellet the debris by centrifugation at 4°C for 10 minutes at $\geq 10,000 \times g$ in a microcentrifuge.
8. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
9. Add 500 μ l of isopropanol to the supernatant. Invert the tube 30-40 times.
10. Pellet the purified RNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.
11. Carefully pour off the isopropanol without dislodging the RNA pellet.
12. Rinse twice with 75% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet.
13. Resuspend the RNA in 10-35 μ l of TE Buffer.
14. Add 1 μ l of RiboGuard RNase Inhibitor (optional).

8. Troubleshooting RNA Purifications

Little or no RNA after resuspension in TE buffer

- 1) **Increase the amount of tissue or biological fluid.** Use the recommended amount of starting material or use the recommended ratio of tissue:lysis buffer as indicated in the protocol. Increase the amount of tissue, particularly if purifying RNA from a biological source other than those listed in the protocols.
- 2) **Increase the efficiency of cell lysis.** Either increase the amount of Proteinase K used during lysis or increase the time of incubation. In addition, vortex during Proteinase K treatment to facilitate lysis. If these adjustments fail, homogenize the tissue to more fully disrupt the cell membrane or wall.
- 3) **Decrease the amount of TE buffer.** Use less TE Buffer to resuspend precipitated RNA.
- 4) **Avoid contamination by exogenous or endogenous nucleases.** Ensure that tissue or biological fluids were properly collected and stored. Use sterile technique. Add ribonuclease inhibitor to the TE Buffer before resuspension.
- 5) **Ensure that RNA remains following isopropanol precipitation.** Make certain that the RNA pellet adheres to the microcentrifuge tube during washing of the pellet with 70% ethanol.

A₂₆₀/A₂₈₀ ratio is too low

- 1) **Decrease the amount of starting material.** The RNA is contaminated with protein. Use less tissue or biological fluid; alternatively, dilute the RNA to 300 µl with Tissue and Cell Lysis Solution, and repeat the purification protocol.

Loose protein pellet

- 1) **Cool sample before protein precipitation.** Cool the sample thoroughly on ice before adding the MPC Protein Precipitation Reagent. If the pellet remains loose, centrifuge again. Carefully decant to minimize transfer of precipitated protein. Note that a small degree of transfer is generally not detrimental.

9. Reference

1. Miller, S.A. *et al.*, (1988) *Nucl. Acids Res.* **16**, 1215.

[†]Covered by U.S. Patent No. 6,270,962, European Patent No. 0742838, German Patent No. DE4411588C1, and other issued or pending applications in the U.S. and other countries that are either assigned or exclusively licensed to Epicentre.

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10. Appendix A

MasterPure™ RNA Purification Kit

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Purification of RNA from Whole Blood: MasterPure Reagent Volume Chart

Whole Blood	Tube Size	Red Cell Lysis Solution	Tissue and Cell Lysis Solution	MPC	Isopropanol
0.2 ml	1.5 ml	0.6 ml	0.3 ml	175 ml	500 ml
0.6 ml	15 ml	1.8 ml	0.9 ml	525 ml	1.5 ml
0.8 ml	15 ml	2.4 ml	1.2 ml	700 ml	2.0 ml
1 ml	15 ml	3.0 ml	1.5 ml	875 ml	2.5 ml
2 ml	15 ml	6.0 ml	3.0 ml	1.75 ml	5.0 ml
3 ml	15 ml	9.0 ml	4.5 ml	2.6 ml	7.5 ml
4 ml	50 ml	12 ml	6.0 ml	3.5 ml	10 ml
5 ml	50 ml	15 ml	7.5 ml	4.4 ml	12.5 ml
6 ml	50 ml	18 ml	9.0 ml	5.3 ml	15 ml
7 ml	50 ml	21 ml	10.5 ml	6.1 ml	17.5 ml
8 ml	50 ml	24 ml	12 ml	7.0 ml	20 ml
9 ml	50 ml	27 ml	13.5 ml	7.9 ml	22.5 ml
10 ml	50 ml	30 ml	15 ml	8.8 ml	25 ml
12 ml	50 ml	36 ml	18 ml	10.5 ml	30 ml

DNase I	DNase I Buffer	2X T & C	MPC	Isopropanol	Ribo-Guard
5 ml	0.2 ml	0.2 ml	0.2 ml	500 ml	1 ml
15 ml	0.6 ml	0.6 ml	0.6 ml	1.5 ml	3 ml
20 ml	0.8 ml	0.8 ml	0.8 ml	2.0 ml	3 ml
25 ml	1 ml	1 ml	1 ml	2.5 ml	3 ml
50 ml	2 ml	2 ml	2 ml	5.0 ml	3 ml
75 ml	3 ml	3 ml	3 ml	7.5 ml	5 ml
100 ml	4 ml	4 ml	4 ml	10 ml	6 ml
125 ml	5 ml	5 ml	5 ml	12.5 ml	6 ml
150 ml	6 ml	6 ml	6 ml	15 ml	8 ml
175 ml	7 ml	7 ml	7 ml	17.5 ml	8 ml
200 ml	8 ml	8 ml	8 ml	20 ml	10 ml
225 ml	9 ml	9 ml	9 ml	22.5 ml	10 ml
250 ml	10 ml	10 ml	10 ml	25 ml	12 ml
300 ml	12 ml	12 ml	12 ml	30 ml	12 ml