Manual

MasterPure Complete DNA and RNA Purification Kit

For Research Use Only. Not for use in diagnostic procedures.

MasterPure™ Complete DNA and RNA Purification Kit is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

MasterPure Complete DNA and RNA Purification Kit

Contents

Manual

MasterPure Complete DNA and RNA Purification Kit

1. Introduction

The MasterPure Complete DNA and RNA Purification Kit provides all of the reagents necessary to recover nucleic acid from a wide variety of biological sources. This kit uses a rapid desalting process[1] to remove contaminating macromolecules, avoiding toxic organic solvents. The purified nucleic acid can be used subsequently in many applications including hybridisation, restriction enzyme digestion and PCR amplification.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
MasterPure Complete DNA and RNA Purification Kit*	200 DNA or 100 RNA purifications	MC85200	RNase-Free DNase I (1 U/µL)	E0013-1D1	500 µL
			RiboGuard™ RNase Inhibitor (40 U/µL)	E0126-40D3	100 µL
			TE Buffer	SS000001-D2	8 mL
			Proteinase K, 50 µg/µL	SS000099-D3	200 µL
			RNase A, 5 µg/µL	SS000213-D4	400 µL
			1X DNase Buffer	SS000270-D2	20 mL
			MPC Protein Precipitation Reagent	SS000399-D3	60 mL
			Red Cell Lysis Solution	SS000400-D2	120 mL
			Tissue and Cell Lysis Solution	SS000401-D2	60 mL
			2X T and C Lysis Solution	SS000402-D3	60 mL
	10 DNA or 5 RNA purifications	MC89010	RNase-Free DNase I (1 U/µL)	E0013-1D7	60 µL
			RiboGuard RNase Inhibitor (40 U/µL)	E0126-40D1	5 µL
			TE Buffer	SS000001-D1	1.2 mL
			Proteinase K, 50 µg/µL	SS000099-D1	10 µL
			RNase A, 5 µg/µL	SS000213-D1	20 µL
			1X DNase Buffer	SS000270-D1	1 mL
			MPC Protein Precipitation Reagent	SS000399-D1	5 mL
			Red Cell Lysis Solution	SS000400-D1	6 mL
			Tissue and Cell Lysis Solution	SS000401-D1	3 mL
			2X T and C Lysis Solution	SS000402-D1	4 mL

* All MasterPure Complete DNA and RNA Purification Kit components are also available separately.

3. Product specifications

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

Storage buffers: 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 $CaCl_2$, 0.1% Triton® X-100 (Rohm & Haas) and 1 mM dithiothreitol; RNase A is supplied in a 50% glycerol solution containing 25 mM sodium acetate (pH 4.6); RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$ and 10 mM $CaCl_2$.

Quality control: The MasterPure Complete DNA and RNA Purification Kit is function-tested by purifying total nucleic acid, DNA and RNA from *E. coli*. Purified nucleic acid quality and yield are assayed by agarose gel electrophoresis, spectrophotometry, fluorimetry and use as a template for PCR.

4. General considerations

1. **Tissue sources:** We have used the kit to isolate nucleic acid from a variety of sources including: bovine liver, human HL-60 tissue culture cells, paraffin-embedded breast tumor tissue (see below), human whole blood and plasma, saliva, mouse tail, corn and geranium leaf, *E. coli* and lambda phage. Tissues other than those mentioned here are likely to be compatible with the kit with some optimisation.
2. **Isolation of DNA from paraffin-embedded tissue:** DNA 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 DNA from embedded tissues that is amenable to PCR, we recommend preserving the tissues in either acetone, 95% ethanol or 95% buffered formalin, with fixation times of less than 24 hours.2 Choose PCR primers such that the resultant amplification products are less than or equal to 300 bp in length. The use of xylene or Hemo-D to extract the paraffin has been shown to increase DNA yields, and an alternate protocol is provided. Generally, we do not recommend using the kit to isolate RNA from embedded tissues, as the RNA in these samples is likely to be substantially degraded. Nevertheless, you can use the kit for that application by following the protocol outlined on page 11.
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 RNA from DNA preparations with RNase A, or the removal of DNA from RNA preparations with RNase-Free DNase I, is unnecessary for many applications. These steps may be eliminated from the protocol depending upon the intended use of the DNA or 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. **Complete RNA removal:** If complete removal of RNA is required for your application, refer to the Complete RNA removal protocol (Part O).

5. Total nucleic acids purification protocol

The following protocol is provided for the purification of total nucleic acids from several biological sources (see General considerations). Lyse the fluid or tissue as outlined in Part A, and then proceed with the remainder of the protocol as outlined in Part B. Additional purification protocols begin on page 12. If complete removal of RNA is required for your application, follow the protocol for Complete removal of RNA (Part O).

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 µL of Proteinase K into 150 µL of 2X T and C Lysis Solution for each sample.
3. Transfer 150 µL of the fluid sample to a microcentrifuge tube, add 150 µL 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 µL of Proteinase K, into 300 µL of Tissue and Cell Lysis Solution for each sample.
2. Pellet cells by centrifugation (0.5-1 x 10^6 mammalian cells; 0.1-0.5 mL of an overnight culture of *E. coli*) and discard the supernatant, leaving approximately 25 µL of liquid.
3. Vortex for 10 seconds to resuspend the cell pellet.
4. Add 300 µL 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 µL of Proteinase K into 300 µL of Tissue and Cell Lysis Solution for each sample.
3. Homogenise fresh tissue or grind frozen tissues in liquid nitrogen and transfer to a microcentrifuge tube.
4. Add 300 µL 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 (Becton Dickinson Corp.). Transfer 200 µL of whole blood into a microcentrifuge tube.
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 10-50 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 nucleic acid precipitation in Part B.

Alternate protocol for formalin-fixed, paraffin-embedded (FFPE) tissues

Note: *This protocol uses xylene or Hemo-D to extract the paraffin. A newer and safer product, Citrusolv, may also be used.*

1. Remove a section of tissue using a clean microtome blade; if possible, trim excess paraffin.
2. Place 10-50 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. Add 1-5 mL of xylene or Hemo-D to extract the paraffin and incubate at room temperature for 10 minutes. Pour off the solvent.
4. Repeat Step 3.
5. Add 1-5 mL of 100% ethanol and incubate at room temperature for 10 minutes. Pour off the ethanol.

6. Repeat Step 5.
7. Aspirate all of the remaining ethanol.
8. Dilute 2 µL of Proteinase K into 300 µL of Tissue and Cell Lysis Solution for each sample, and mix.
9. Add 300 µL of Tissue and Cell Lysis Solution containing the Proteinase K to the sample and mix.
10. Incubate at 65 °C for 30 minutes; followed by a brief (10 seconds) vortex mix.
11. Place the samples on ice for 3-5 minutes and then proceed with total nucleic acid precipitation in Part B.

B. Precipitation of total nucleic acids (for all biological samples)

1. Add 150 µL of MPC Protein Precipitation Reagent to 300 µL of lysed sample and vortex vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4 °C for 10 minutes at ≥10,000 x g in a microcentrifuge. If the resultant pellet is clear, small or loose, add an additional 25 µL of MPC Protein Precipitation Reagent, mix and pellet the debris again.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 µL 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 pellet.
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 pipette.
8. Resuspend the total nucleic acids in 35 µL of TE Buffer.

6. DNA purification protocols

The following protocol is provided for the purification of DNA from several biological sources (see General considerations). Lyse the fluid or tissue as outlined in Part C, and then proceed with the remainder of the protocol as outlined in Part D. Additional purification protocols begin on page 12. If complete removal of RNA is required for your application, follow the protocol for Complete removal of RNA (Part O).

C. 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 µL of Proteinase K into 150 µL of 2X T and C Lysis Solution for each sample.
3. Transfer 150 µL of the fluid sample to a microcentrifuge tube, add 150 µL 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. Cool the samples to 37 °C and add 1 µL of 5 µg/mL RNase A to the sample; mix thoroughly.
6. Incubate at 37 °C for 30 minutes.
7. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation in Part D.

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

1. Dilute 1 µL of Proteinase K into 300 µL of Tissue and Cell Lysis Solution for each sample.
2. Pellet cells by centrifugation (0.5-1 x 10^6 mammalian cells; 0.1-0.5 mL of an overnight culture of *E. coli*) and discard the supernatant, leaving approximately 25 µL of liquid.
3. Vortex for 10 seconds to resuspend the cell pellet.
4. Add 300 mL 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. Cool the samples to 37 °C and add 1 µL of 5 mg/mL RNase A to the sample; mix thoroughly.
7. Incubate at 37 °C for 30 minutes.
8. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation in Part D.

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 µL of Proteinase K into 300 µL of Tissue and Cell Lysis Solution for each sample.
3. Homogenise fresh tissue or grind frozen tissues in liquid nitrogen and transfer to a microcentrifuge tube.
4. Add 300 µL 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. Cool the samples to 37 °C and add 1 µL of 5 µg/µL RNase A to the sample; mix thoroughly.
7. Incubate at 37 °C for 30 minutes.
8. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation in Part D.

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.
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 brieffvortexing.
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. Add 1 µL of RNase A and mix thoroughly.
8. Incubate at 37 °C for 30 minutes.
9. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation in Part D.

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 10-50 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. Cool the samples to 37 °C and add 1 µL of RNase A to the sample; mix thoroughly.
7. Incubate at 37 °C for 30 minutes.
8. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation in Part D.

Alternate protocol for formalin-fixed, paraffin-embedded (FFPE) tissues

Note: *This protocol uses xylene or Hemo-D to extract the paraffin. A newer and safer product, Citrusolv, may also be used.*

1. Remove a section of tissue using a clean microtome blade; if possible, trim excess paraffin.
2. Place 10-50 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. Add 1-5 mL of xylene or Hemo-D to extract the paraffin and incubate at room temperature for 10 minutes. Pour off the solvent.
4. Repeat Step 3.
5. Add 1-5 mL of 100% ethanol and incubate at room temperature for 10 minutes. Pour off the ethanol.
6. Repeat Step 5.
7. Aspirate all of the remaining ethanol.
8. Dilute 2 µL of Proteinase K into 300 µL of Tissue and Cell Lysis Solution for each sample, and mix.
9. Add 300 µL of Tissue and Cell Lysis Solution containing the Proteinase K to the sample and mix.
10. Incubate at 65 °C for 30 minutes; followed by a brief (10 seconds) vortex mix.
11. Cool the samples to 37 °C and add 1 µL of RNase A to the sample; mix thoroughly.
12. Incubate at 37 °C for 30 minutes.
13. Place the samples on ice for 3-5 minutes and then proceed with DNA precipitation in Part D.

D. Precipitation of total nucleic acids (for all biological samples)

1. Add 150 µL of MPC Protein Precipitation Reagent to 300 µL of lysed sample and vortex vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4 °C for 10 minutes at ≥10,000 x g in a microcentrifuge. If the resultant pellet is clear, small or loose, add an additional 25 µL of MPC Protein Precipitation Reagent, mix and pellet the debris again.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 µL of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
5. Pellet the DNA by centrifugation at 4 °C for 10 minutes in a microcentrifuge.

6. Carefully pour off the isopropanol without dislodging the DNA pellet.
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 pipette.
8. Resuspend the DNA in 35 µL of TE Buffer.

7. RNA purification protocols

The following protocol is provided for the purification of RNA from several biological sources (see General considerations). Lyse the fluid or tissue as outlined in Part E, and then proceed with the remainder of the protocol as outlined in Part F. If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined in Part G. Use appropriate techniques to minimise degradation by exogenous ribonucleases. Additional purification protocols begin on page 12.

E. 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 µL of Proteinase K into 150 µL of 2X T and C Lysis Solution for each sample.
3. Transfer 150 µL of the fluid sample to a microcentrifuge tube, add 150 µL 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 F.

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

1. Dilute 1 µL of Proteinase K into 300 µL of Tissue and Cell Lysis Solution for each sample.
2. Pellet cells by centrifugation (0.5-1 x 10^6 mammalian cells; 0.1-0.5 mL of an overnight culture of *E. coli*) and discard the supernatant, leaving approximately 25 µL of liquid.
3. Vortex for 10 seconds to resuspend the cell pellet.
4. Add 300 µL of Tissue and Cell Lysis Solution containing the Proteinase K and mixthoroughly.
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 F.

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 µL of Proteinase K into 300 µL of Tissue and Cell Lysis Solution for each sample.
3. Homogenise fresh tissue or grind frozen tissues in liquid nitrogen and transfer to a microcentrifuge tube.
4. Add 300 µL 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 F.

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.
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 F.

Formalin-fixed, paraffin-embedded (FFPE) tissues

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 nucleic acid precipitation in Part F.

F. Precipitation of total nucleic acids (for all biological samples)

1. Add 150 µL of MPC Protein Precipitation Reagent to 300 µL of lysed sample and vortex vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4 °C for 10 minutes at ≥10,000 x g in a microcentrifuge. If the resultant pellet is clear, small or loose, add an additional 25 µL of MPC Protein Precipitation Reagent, mix and pellet the debris again.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 µL 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 G. Otherwise, complete the remainder of Part F.

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 pipette.
8. Resuspend the total nucleic acids in 35 µL of TE Buffer.

G. Removal of contaminating DNA from total nucleic acids preparations (for all biological samples)

1. Remove all of the residual isopropanol with a pipette.
2. Prepare 200 µL of DNase I solution for each sample by diluting 5 µL of RNase-Free DNase I up to 200 µL with 1X DNase Buffer.
3. Completely resuspend the total nucleic acid pellet in 200 µL 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 µL of 2X T and C Lysis Solution; vortex for 5 seconds.
6. Add 200 µL 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 ≥10,000 x g in a microcentrifuge.
8. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
9. Add 500 µL 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 pipette.
13. Resuspend the RNA in 10-35 µL of TE Buffer.
14. Add 1 µL of RiboGuard RNase Inhibitor (optional).

8. Additional purification protocols

The following protocol is provided for the purification of total nucleic acid, DNA or RNA from plasma. Lyse the plasma as outlined in Part H; if isolating DNA, users may add an optional RNase A step following treatment with Proteinase K (see DNA purification protocols). Precipitate the nucleic acid as outlined in Part I. If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined in Part N. For the purification of RNA, use appropriate techniques to minimise degradation by exogenous ribonucleases.

H. Lysis of plasma

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

1. Collect plasma samples. Transfer 50 µL of plasma into a microcentrifuge tube.
2. Dilute 1 µL of 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 I.

I. Precipitation of total nucleic acids (from plasma lysis)

1. Place the samples on ice for 5 minutes.
2. Add 250 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 ≥10,000 x 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 N. Otherwise, complete the remainder of Part I.
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 pipette.
9. Resuspend the nucleic acid in 35 µL of TE Buffer.

The following protocol is provided for the purification of total nucleic acids, DNA or RNA from whole blood without the initial lysis of the red blood cells. Lyse the blood as outlined in Part J; if isolating DNA, users may add an optional RNase A step following treatment with Proteinase K (see DNA purification protocols). If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined in Part N. For the purification of RNA, use appropriate techniques to minimise degradation by exogenous ribonucleases.

J. 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 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 K.

K. 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 ≥10,000 x 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 N. Otherwise, complete the remainder of Part K.

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 pipette.
9. Resuspend the total nucleic acids in 35 µL of TE Buffer.

The following protocol is provided for the purification of total nucleic acids, DNA or RNA from buffy coat of blood. Prepare buffy coat and lyse the white cells as outlined in Part L, and then proceed with precipitation of total nucleic acids as described in Part M. If further purification of RNA (to remove contaminating DNA) is required, follow the protocol outlined in Part N. For the purification of RNA, use appropriate techniques to minimise degradation by exogenous ribonucleases.

L. 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. The 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 M.

M. 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 ≥10,000 x 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 N. Otherwise, complete the remainder of Part M.

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 pipette.
8. Resuspend the total nucleic acids in 35 µL of TE Buffer.

N. Removal of contaminating DNA from RNA preparations

1. Remove all of the residual isopropanol with a pipette.
2. Prepare 200 µL of DNase I solution for each sample by diluting 10 µL of RNase-Free DNase I up to 200 µL with 1X DNase Buffer.
3. Completely resuspend the total nucleic acids pellet in 200 µL of DNase I solution.
4. Incubate at 37 °C for 30 minutes.
5. Add 200 µL of 2X T and C Lysis Solution; vortex for 5 seconds.
6. Add 200 µL of MPC Protein Precipitation Reagent; vortex 10 seconds; place on ice for 3-5 minutes.
7. Pellet the debris by centrifugation for 10 minutes at ≥10,000 x g in a microcentrifuge.
8. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
9. Add 500 µL 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 pipette.
13. Resuspend the RNA in 10-35 µL of TE Buffer.
14. Add 1 µL of RiboGuard RNase Inhibitor (optional).

O. Complete removal of RNA

1. Add 1 µL of RNase A to the sample; mix thoroughly.
2. Incubate at 37 °C for 30 minutes.
3. Add 14 µL TE Buffer and 50 µL of 2X T and C Lysis Solution to each sample.
4. Place the samples on ice for 3-5 minutes. Add 100 µL of MPC Protein Precipitation Reagent and mix by vortexing vigorously for 10 seconds.
5. Pellet the debris by centrifugation at 4 °C for 10 minutes at ≥10,000 x g in a microcentrifuge.
6. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
7. Add 200 µL of isopropanol to the recovered supernatant. Invert the tube several (30-40) times.
8. Pellet the DNA by centrifugation at 4 °C for 10 minutes in a microcentrifuge.
9. Carefully pour off the isopropanol without dislodging the DNA pellet.
10. 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 pipette.
11. Resuspend the DNA in 35 µL of TE Buffer.

9. Troubleshooting nucleic acid purifications

Little or no nucleic acid 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 nucleic acids 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, homogenise 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 nucleic acids.
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 nucleic acids remain following isopropanol precipitation.** Make certain that the nucleic acid pellet adheres to the microcentrifuge tube during washing of the pellet with 70% ethanol.

A_{260}/A_{280} ratio is too low

1) **Decrease the amount of starting material.** The nucleic acid is contaminated with protein. Use less tissue or biological fluid; alternatively, dilute the nucleic acid to 300 µL with Tissue and Cell Lysis Solution and follow the protocol for Total nucleic acid purification.

A_{260}/A_{280} ratio is too high

1) **Treat with ribonuclease.** The DNA is contaminated with RNA. If RNase A treatment was omitted, treat with RNase A. Note that precipitation of nucleic acid is extremely efficient, resulting in the precipitation of small oligomers of ribonucleotides. If these are undesirable, treat the DNA with RNase I (available separately) to degrade these oligomers and precipitate the DNA.

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 minimise transfer of precipitated protein. Note that a small degree of transfer is generally not detrimental.

DNA rehydrates slowly

1) **Decrease drying time.** Remove residual ethanol with a pipette and air dry briefly. Suspend in TE Buffer and disrupt the DNA pellet gently with a pipette. If necessary, the DNA may be left at room temperature overnight to rehydrate. Use additional TE Buffer as required.

Residual RNA in DNA preparations

1) Remove RNA. If complete removal of RNA is required

10. References

1. Miller, S.A. *et al.* (1988) *Nucl. Acids Res.* **16,** 1215.
2. Shimizu, H. and Burns, J.C. (1995) in: PCR Strategies, Innis, M.A. *et al.*, (eds.), Academic Press, San Diego, 2.

11. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Integrated tools.
Accelerated science.

@LGCBiosearch | biosearchtech.com

GEN/900/EK/0721

For Research Use Only. Not for use in diagnostic procedures.

bsi. ISO 13485 Medical Devices Quality Management
FM 598408

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC