Manual

MasterPure DNA Purification Kit for Blood Version II

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

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Manual

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1. Introduction

The MasterPure DNA Purification Kit for Blood Version II provides all of the reagents necessary to recover nucleic acid from whole blood or buffy coat. 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, next-generation sequencing and PCR amplification.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part number	Volume
MasterPure DNA Purification Kit for Blood Version II	For 400 mL of whole blood	MB711400	TE Buffer	MTE0970	70 mL
			RNase A (5 µg/µL)	MRNA092	2 mL
			MPC Protein Precipitation Reagent	MMP095H	500 mL
			Red Cell Lysis Solution	MRC0912H-1	2 × 600 mL
			Tissue & Cell Lysis Solution	MTC096H	600 mL

3. Product specifications

Storage: Store the RNase A at -20 °C in a freezer without a defrost cycle. Store the remainder of the kit at room temperature.

Storage buffer: RNase A is supplied in a 50% glycerol solution containing 25 mM sodium acetate (pH 4.6).

Quality control: The MasterPure DNA Purification Kit for Blood Version II is function-tested by extracting DNA using the whole blood protocol. DNA quality and yield are assayed by spectrophotometry, agarose gel electrophoresis and use as a template for PCR.

4. DNA purification protocols

A. Buffy coat DNA purification protocol

1. Draw 5 mL of blood into an EDTA Vacutainer® tube (Becton Dickinson Corp.); separate fractions by centrifugation at 1,000 x g for 15 minutes. Draw 5 mL of blood into an EDTA Vacutainer® tube (Becton Dickinson Corp.); separate fractions by centrifugation at 1,000 x g for 15 minutes.
2. Carefully transfer 600 µL of buffy coat (the white interface between the plasma and the red blood cells) to a new tube.

   Note: *The transfer of some red blood cells is not detrimental to the purification of DNA from buffy coat. To maximise yields, process samples through lysis (Step 8). If samples must be stored before lysis, place at 4 °C for 1-7 days; or for a longer term, at -20 °C (The yield of DNA may be decreased if samples are stored before Step 8).*

3. Vortex the buffy coat sample. Split the sample by transferring 300 µL of the sample to **each** of two 1.5 mL microcentrifuge tubes. Process the two tubes in parallel.
4. Add 1.2 mL of Red Cell Lysis Solution to each tube. Invert the tubes three times to mix, and then flick the bottom of the tubes to resuspend any remaining material.
5. Incubate at room temperature for 5 minutes; invert three times to mix and then flick the tubes as outlined above. Continue incubating at room temperature for an additional 5 minutes; invert three times to mix and then flick the tubes.
6. Pellet the white blood cells by centrifugation for 25 seconds in a microcentrifuge.
7. Remove most of the supernatant, leaving ~25 µL of liquid. Vortex to suspend the pellet.
8. Resuspend the white blood cells in 600 µL of Tissue and Cell Lysis Solution by pipetting several times. The samples may be stored for several months at room temperature.
9. Add 250 µL of the MPC Protein Precipitation Reagent and vortex vigorously for ≥30 seconds.
10. Pellet the debris by centrifugation for 10 minutes at ≥10,000 x g in a microcentrifuge.
11. Pour the supernatant into a clean microcentrifuge tube and add 700 µL of isopropanol. Mix by inverting the tube 30-40 times; a stringy precipitate should be visible.
12. Pellet the DNA by centrifugation at 4 °C for 10 minutes in a microcentrifuge.
13. Carefully pour off the supernatant without dislodging the pellet. 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.
14. Resuspend the DNA in 200 µL of TE Buffer; incubate overnight at room temperature. Alternatively, resuspend the DNA by pipetting repeatedly followed by vortexing for 10 seconds. Store the purified DNA at -20 °C.
15. Quantitate the DNA by electrophoresis, spectrophotometry or fluorimetry. The concentration should be approximately 200-500 µg/mL.

B. Whole-blood DNA purification protocols

Two protocols are provided for the purification of total genomic DNA from human blood:

1) DNA Purification for 5 mL of Whole Blood (with RBC Lysis)
2) DNA Purification for 200 µL of Whole Blood (with RBC Lysis)

Refer to Chart 1 to scale reagent volumes if starting with other amounts of whole blood.

Whole blood	Tube size	RBC Lysis	T & C	RNase A	MPC	Isopropanol	70% ethanol
0.2 mL	1.5 mL	0.6 mL	0.3 mL	1 µL	175 µL	500 µL	500 µL
0.6 mL	15 mL	1.8 mL	0.9 mL	3 µL	525 µL	1.5 mL	500 µL
0.8 mL	15 mL	2.4 mL	1.2 mL	4 µL	700 µL	2.0 mL	500 µL
1 mL	15 mL	3.0 mL	1.5 mL	5 µL	875 µL	2.5 mL	500 µL
2 mL	15 mL	6.0 mL	3.0 mL	10 µL	1.75 mL	5.0 mL	500 µL
3 mL	15 mL	9.0 mL	4.5 mL	15 µL	2.6 mL	7.5 mL	500 µL
4 mL	50 mL	12 mL	6.0 mL	20 µL	3.5 mL	10 mL	500 µL
5 mL	50 mL	15 mL	7.5 mL	25 µL	4.4 mL	12.5 mL	500 µL
6 mL	50 mL	18 mL	9.0 mL	30 µL	5.3 mL	15 mL	1 mL
7 mL	50 mL	21 mL	10.5 mL	35 µL	6.1 mL	17.5 mL	1 mL
8 mL	50 mL	24 mL	12 mL	40 µL	7.0 mL	20 mL	1 mL
9 mL	50 mL	27 mL	13.5 mL	45 µL	7.9 mL	22.5 mL	2 mL
10 mL	50 mL	30 mL	15 mL	50 µL	8.8 mL	25 mL	2 mL
12 mL	50 mL	36 mL	18 mL	60 µL	10.5 mL	30 mL	2 mL
14 mL (2 x 7 mL)	2 x 50 mL	42 mL (2 x 21 mL)	21 mL (2 x 10.5 mL)	70 µL (2 x 35 µL)	12 mL (2 x 6 mL)	35 mL (2 x 17.5 mL)	4 mL (2 x 2 mL)
15 mL (2 x 7.5 mL)	2 x 50 mL	45 mL (2 x 22.5 mL)	22.5 mL (2 x 11.3 mL)	75 µL (2 x 37.5 µL)	13 mL (2 x 6.5 mL)	38 mL (2 x 19 mL)	4 mL (2 x 2 mL)
20 mL (2 x 10 mL)	2 x 50 mL	60 mL (2 x 30 mL)	30 mL (2 x 15 mL)	100 µL (2 x 50 µL)	18 mL (2 x 9 mL)	50 mL (2 x 25 mL)	4 mL (2 x 2 mL)

Table 1. Purification of DNA from whole blood: MasterPure reagent volume.

B1. DNA purification for 5 mL of whole blood (with RBC Lysis)

Expected yield: 75-225 µg of DNA

1. Draw 5 mL of blood into an EDTA Vacutainer tube. Transfer 5 mL of whole blood into a 50 mL centrifuge tube.
2. Add 15 mL 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 centrifuge.
5. Remove most of the supernatant, leaving approximately 500 µL of liquid. Vortex to suspend the pellet.
6. Resuspend the white blood cells in 7.5 mL of Tissue and Cell Lysis Solution by pipetting several times.
7. Add 25 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 to precipitation of total DNA.
10. Add 4.4 mL of MPC Protein Precipitation Reagent to 7.5 mL of lysed sample and vortex vigorously for 10 seconds.
11. Pellet the debris by centrifugation for 10 minutes at ≥10,000 x g in a centrifuge.
12. Transfer the supernatant to a clean centrifuge tube and discard the pellet.
13. Add 12.5 mL of isopropanol to the recovered supernatant. Mix by inverting the tube 30-40 times.
14. Pellet the DNA by centrifugation at 4 °C for 10 minutes in a centrifuge.
15. Carefully remove the isopropanol using a pipette, without dislodging the DNA pellet.
16. 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.
17. Resuspend the DNA in 500 µL of TE Buffer. Quantitate the DNA by electrophoresis, spectrophotometry or fluorimetry.

B2. DNA purification for 200 µL of whole blood (with RBC Lysis)

Expected yield: 3-9 µg of DNA

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 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 to precipitation of total DNA.
10. Add 175 µL of MPC Protein Precipitation Reagent to 300 µL of lysed sample and vortex vigorously for 10 seconds.
11. Pellet the debris by centrifugation for 10 minutes at ≥10,000 x g in a microcentrifuge.
12. Transfer the supernatant to a clean centrifuge tube and discard the pellet.
13. Add 500 µL of isopropanol to the recovered supernatant. Mix by inverting the tube 30-40 times.
14. Pellet the DNA by centrifugation at 4 °C for 10 minutes in a microcentrifuge.
15. Carefully remove the isopropanol using a pipette, without dislodging the DNA pellet.
16. 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.

17. Resuspend the DNA in 35 µL of TE Buffer. Quantitate the DNA by electrophoresis, spectrophotometry or fluorimetry.

5. Reference

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

6. Further support

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

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