epicentre®
an Illumina® company

MasterPure™ DNA Purification Kit for Blood Version II

Cat. Nos. MB711740 and MB711400

Connect with Epicentre on our blog (**epicentral.blogspot.com**),
Facebook (**facebook.com/EpicentreBio**), and Twitter (**@EpicentreBio**).

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 hybridization, restriction enzyme digestion, and PCR amplification. 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 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.

3. Kit Contents and Specifications

Cat. #	Concentration	Quantity
MasterPure™ DNA Purification Kit for Blood Version II Contents		
The MasterPure DNA Purification Kit for Blood Version II is available in two sizes, sufficient to purify DNA from 40 ml and 400 ml of whole blood.		
For 40 ml Whole Blood		
MB711740		
Red Cell Lysis Solution		120 ml
Tissue and Cell Lysis Solution		60 ml
MPC Protein Precipitation Reagent		36 ml
RNase A	@ 5 µg/µl	200 µl
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)		7 ml
For 400 ml Whole Blood		
MB711400		
Red Cell Lysis Solution		1,200 ml
Tissue and Cell Lysis Solution		600 ml
MPC Protein Precipitation Reagent		360 ml
RNase A	@ 5 µg/µl	2 ml
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)		70 ml

All kit components are available separately.

4. Related Products

The following products are also available:

- MasterPure™ Complete DNA and RNA Purification Kits
- MasterPure™ DNA Purification Kit
- MasterPure™ RNA Purification Kit
- MasterAmp™ Buccal Swab DNA Extraction Kits
- BuccalAmp™ DNA Extraction Kits
- MasterAmp™ PCR Optimization Kits
- MasterAmp™ *Taq, Tth, Tfl*, and AmpliTherm™ DNA Polymerases
- FailSafe™ PCR System

5. DNA Purification Protocols

Two protocols are provided for the purification of total genomic DNA from human blood. The buffy coat protocol is recommended when 5 ml of blood is available; the whole blood protocol is recommended when less material is available.

A. Buffy Coat DNA Purification Protocol

1. Draw 5 ml of blood into an EDTA Vacutainer® tube; 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 maximize yields, process samples through lysis (Step 7). 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 7).*
3. Vortex the buffy coat sample. Transfer 300 µl of the sample to two 1.5 ml microcentrifuge tubes and add 1.2 ml of Red Cell Lysis Solution. Invert three times to mix and then flick the bottom of the tube to suspend any remaining material.
4. 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.
5. Pellet the white blood cells by centrifugation for 25 seconds in a microcentrifuge.
6. Remove most of the supernatant, leaving ~25 µl of liquid. Vortex to suspend the pellet.
7. 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.
8. Add 250 µl of the MPC Protein Precipitation Reagent and vortex vigorously for ≥30 seconds.
9. Pellet the debris by centrifugation for 10 minutes at ≥10,000 x g in a microcentrifuge.

10. 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.
11. Pellet the DNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.
12. 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 pipet.
13. 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.
14. Quantitate the DNA by electrophoresis, spectrophotometry, or fluorimetry. The concentration should be approximately 200-500 µg/ml.

B. Whole-Blood DNA Purification Protocols

1) DNA Purification for 5 ml of Whole Blood (with RBC Lysis)
2) DNA Purification for 200 µl of Whole Blood (with RBC Lysis)

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 pour off the isopropanol 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 pipet.
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 pour off the isopropanol 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 pipet.
17. Resuspend the DNA in 35 µl of TE Buffer. Quantitate the DNA by electrophoresis, spectrophotometry, or fluorimetry.

Purification of DNA from Whole Blood: MasterPure™ Reagent Volume Chart

Whole Blood	Tube Size	RBC Lysis	T & C	RNase A	MPC	Isopro-panol	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)

6. Reference

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

Visit our technical blog: **epicentral.blogspot.com**