Montreal Neurological Institute and Hospital
McGill University

CBIG-02-005

DNA EXTRACTION FROM WHOLE BLOOD (CONVENTIONAL METHOD)

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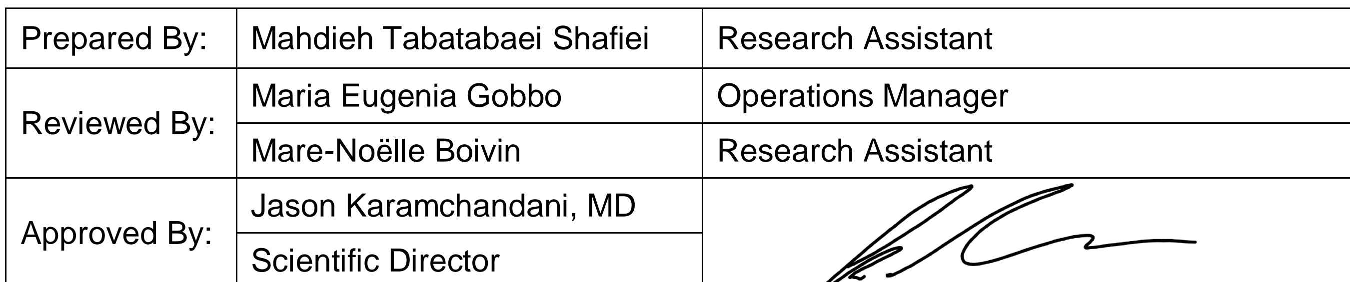

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1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	2-Oct-2020

2. SCOPE AND APPLICATION

This protocol should be used as a secure method of purifying genomic DNA from whole blood. The conventional method is optimized for samples that **HAVE NOT** been stored under optimal conditions, meaning upon blood draw, blood tubes have been stored more than one (1) day at room temperature or more than five (5) days at 4°C degrees or frozen at -80°C degrees for more than one (1) year. For a more rapid procedure for samples that have been stored in optimal conditions, consult SOP *CBIG-02-004 DNA Extraction from Whole Blood (Accelerated Method)*

Should the storage conditions be unknown, please refrain from using the SOP *CBIG-02-004 DNA Extraction from Whole Blood (Accelerated Method)* and continue with this SOP.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

3.1 Reference to Other C-BIG SOPs or Documents

1. CBIG Repository: CBIG-P-0002 DNA Extraction from Whole Blood (Archived)
2. CBIG Repository: CBIG-02-004 DNA Extraction from Whole Blood (Accelerated Method)
3. CBIG Repository: CBIG-02-007 DNA Quantification through NanoDrop Spectrophotometry

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3.2 Reference to External SOPs or Documents

1. Experimental Therapeutics Program: ETP-P-0003 DNA Extraction from Whole Blood
2. Gentra Puregene Handbook, 4th edition Purification of archive-quality DNA

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who extract DNA from whole blood. All personnel who read this SOP should sign the form found in the reading log binder.

5. ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
C-BIG	Clinical Biological Imaging and Genetics Repository
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
Min	Minutes
mL	Milliliter
PTT	Purple/Lavender Top vacutainer
QA	Quality Assurance
QC	Quality Control
RT	Room Temperature
SOP	Standard Operating Procedure
μL	Microliter
°C	Degrees Celsius

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6. MATERIALS AND EQUIPMENT

The materials and equipment listed below are recommendations only and may be substituted by alternative/equivalent products more suitable for site-specific task or procedure.

NOTE: All disposable items are sterile (gamma-irradiated) unless otherwise specified. All equipment, disposables or reagents can be substituted with equivalent materials following evaluation and approval, unless specified otherwise.

NOTE: All sample contact materials must be suitable for RNA work (i.e. RNAse-free). Always use clean gloves to prevent inadvertent RNAse contamination during processing.

Material/Equipment	Material/Equipment (site specific)
Blood collection tube with K2 EDTA, 10 mL, purple/lavender top	BD Diagnostic; Cat # 366643
Cryovials, 1 mL, conical bottom	Nunc Biobanking and Cell Culture Cryogenic Tubes, Thermo Scientific; Cat # 366656
Disposable serological pipets, 5 mL	
Disposable serological pipets, 10 mL	
Filtered pipet tips, 100-1000 µL	
Polypropylene conical tubes, 50 mL	
Polypropylene conical tubes, 15 mL	
Flip cap polypropylene conical tubes, 50 mL	Thermo Scientific, Nunc EZ Flip Cap, 50 mL; Cat # 362696

Vortex	
Micropipet, range 100 μL to 1000 μL	
Clean workstation	
Pipet-Aid	
RBC Lysis Solution	Qiagen, Cat # 158904
Cell Lysis Solution	Qiagen, Cat # 158908
DNA Hydration Solution	Qiagen, Cat # 158916
Protein Precipitation Solution	Qiagen, Cat # 158912
2-Pronanol	
70% EtOH	
Centrifuge with swinging bucket	Centrifuge 5810, Cat # 022625004; with rotor S-4-104, Cat # 5820759003
4°C fridge	
-80°C freezer	
Water Bath	

7. PROCEDURES

PRECAUTIONS: All biological samples derived from human source are considered to be biohazardous. Use appropriate precautions when working with such samples (i.e. personal protection equipment such as gloves, lab coat and safety glasses). All waste (samples and related contact materials) must be placed in marked biohazardous waste containers and disposed of under hospital guidelines.

NOTE: Best results are obtained when whole blood is processed promptly. When possible, processing should be done the same day as the blood draw. If the blood is not

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processed right after blood draw, the vacutainers should be put on the shaker until further processing.

NOTE: If the blood cannot be processed on the same day, it can be placed at RT and processed within five (5) days. The sample needs to be placed on the shaker for 30 min prior to processing.

NOTE: If the blood cannot be processed within five (5) days, see section 7.3

7.1 Extraction of Human DNA from (10 mL ≤ Volume of Whole Blood)

1. Pour 30 mL of RBC Lysis Solution into a 50 mL flip cap tube
2. Pour blood from one (1) PTT into tube with RBC Lysis Solution.
3. Incubate on shaker for 5-10 minutes.
4. Centrifuge at RT for 2 min at 2000Xg with a centrifuge with swinging buckets and discard supernatant.
5. Vortex to briefly re-suspend the pellet in remaining volume.
6. Immediately add 10 mL of Cell Lysis Solution followed by 10 seconds of vortex to mix the solution.

NOTE: Usually no incubation is required; however, if cell clumps are visible, incubate at 37°C until the solution is homogeneous.

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NOTE: At this stage the samples are stable and can be stored at RT for up to two (2) years without jeopardizing the DNA quality.

7. Add 3.33 mL of Protein Precipitation Solution.
8. Vortex vigorously for 20 seconds on high speed.

NOTE: It is important to keep vortexing to a minimum as excessive vortexing can shear DNA strands.

9. Centrifuge for 5 min at 2000Xg.

NOTE: The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation step above.

10. Prepare a new 50 mL polypropylene conical tube with 10 mL of 2-propanol.
11. Pour the supernatant into the conical tube containing the 2-propanol.
12. Mix tube by gently inverting until DNA is visible as threads or a clump, continue to mix until it gets compact.
13. Centrifuge for 3 min at 2000Xg.
14. Carefully discard the supernatant, taking care that the pellet remains in the tube.
15. Wash the pellet with 10 mL of 70% EtOH.
16. Centrifuge for 1 min at 2000Xg and discard supernatant.

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17. Air dry the pellet for 5-10 min. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
18. Add 1 mL DNA of Hydration Solution to the pellet.
19. Store DNA at 4°C and be sure to aliquot (and quantify if needed) within seven (7) days.

NOTE: If the DNA from the same donor and collected at the same time was processed in more than one tube, pool the DNA at this step prior to aliquoting. If doubts exist about the quality being different between the preparations, keep each preparation separate and identify each tube appropriately.

NOTE: Should DNA quantification be needed, see SOP *CBIG-02-007 DNA Quantification through NanoDrop Spectrophotometry*.

20. Aliquot the dissolved DNA in cryovials of 200 µL per vial.
21. DNA vials can be stored at -80°C freezer for long term storage.

7.2 Extraction of Human DNA from Whole Blood (≥ 1 mL and ≤3 mL)

NOTE: If less than one (1) mL of blood is collected in a 10 mL capacity EDTA vacutainer, the EDTA concentration mixed with the whole blood will be too high resulting in unreliable extraction. If this is the case, avoid processing the sample when the volume is less than one (1) mL. Should you require a lower volume of blood, consider using a smaller (5 mL capacity) EDTA vacutainer.

1. Pour 9 mL of RBC lysis solution into a 50 mL flip cap tube and pour blood from PTT.
2. Incubate on shaker for 5 minutes.
3. Centrifuge at RT for 2 min at 2000Xg with centrifuge with swinging bucket and discard supernatant.
4. Re-suspend the pellet in remaining volume using vortex.
5. Immediately add 3 mL of Cell Lysis Solution and vortex briefly to mix

NOTE: Usually no incubation is required; however, if cell clumps are visible, incubate at 37°C until the solution is homogeneous.

NOTE: At this stage the samples are stable and can be stored at RT for up to two (2) years without jeopardizing the DNA quality.

6. Add 1 mL of Protein Precipitation Solution and vortex vigorously for 10 seconds.

NOTE: It is important to keep vortexing to a minimum as excessive vortexing can shear DNA strands.

7. Centrifuge for 5 min at 2000Xg.
8. Prepare a new 50 mL polypropylene conical tube with 3 mL of 2-propanol.
9. Pour the supernatant into the conical tube containing the 2-propanol.

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10. Mix tube by gently inverting until white DNA becomes visible as threads or clumps, continue to mix until it gets compact.
11. Centrifuge for 3 min at 2000Xg.
12. Carefully discard the supernatant, taking care that the pellet remains in the tube.
13. Wash the pellet with 3 mL of 70% EtOH.
14. Centrifuge for 1 min at 2000Xg.
15. Discard supernatant.
16. Air dry the pellet for 5-10 min. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.
17. Add 400 µL of DNA Hydration Solution to the pellet.
18. Store DNA at 4°C and be sure to aliquot (and quantify if needed) within seven (7) days.

<u>NOTE:</u> Should DNA quantification be needed, see SOP *CBIG-02-007 DNA Quantification through NanoDrop Spectrophotometry*.

19. Aliquot the dissolved DNA in cryovials of 200 µL per vial.
20. DNA vials can be stored at -80°C freezers.

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7.3 Freezing Blood

NOTE: If the blood cannot be processed within five (5) days, vacutainers can be frozen down until ready to process.

1. Place the vacutainer in a -80°C freezer. The tubes can remain frozen for up to one (1) year.
2. Once ready to process, allow the vacutainer to gradually thaw for 30 min at RT. Avoid placing the frozen vacutainer directly from -80°C freezer to water bath.
3. Thaw the vacutainer by placing it in the water bath set at 37°C for 20 min. Halfway through the incubation, invert the vacutainer gently.
4. Continue the extraction with the steps from 1-20 of section 7.1.

7.4 Summary Table

Steps		
No.	**≥ 1 mL and ≤3 mL**	**≤ 10mL**
1	Add blood to 9 mL of RBC Lysis Buffer	Add blood to 30 mL of RBC Lysis Buffer
2	Incubate at RT on shaker for 5 min	
3	Centrifuge for 2 min at 2000Xg and discard supernatant	
4	Re-suspend pellet with a vortex	
5	Add 3 mL of Cell Lysis Solution	Add 10 mL of Cell Lysis Solution
6	vortex for 10-20 seconds to mix	
7	Add 1 mL of Protein Precipitation Solution	Add 3.33 mL of Protein Precipitation Solution
8	Vortex intermittently	
9	Centrifuge for 5 min at 2000Xg	

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10	Pour supernatant into a new tube containing 3 mL of 2-propanol	Pour supernatant into a new tube containing 10 mL of 2-propanol
11	Mix gently by inverting tube until DNA fibers are visible	
12	Centrifuge for 3 min at 2000Xg and discard the supernatant	
13	Wash the pellet with 3 mL of 70% EtOH	Wash the pellet with 10 mL of 70% EtOH
14	Centrifuge for 1 min at 2000Xg and discard the supernatant	
15	Air-dry pellet	
16	Add 400 µL of DNA Hydration Solution	Add 1 mL of DNA Hydration Solution
17	Store the DNA at 4°C until ready to quantify (optional) and aliquot	
18	Transfer DNA to long term storage in -80°C freezer	

8. QUALITY CONTROL / QUALITY ASSURANCE

All the equipment used should be monitored, cleaned and calibrated per their specific SOPs.

Reagents with an expiry date should be monitored and used before this date. If used after expiry date, it should be recorded.

9. APPENDICES/FORM

9.1 Appendix A – Sample processing form – DNA Extraction from Whole Blood (Conventional Method)

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