Montreal Neurological Institute and Hospital
McGill University

CBIG-02-006

DNA EXTRACTION FROM SALIVA

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1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	01-May-2020

2. SCOPE AND APPLICATION

This protocol is to be used for the extraction of DNA from saliva.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference *C-BIG Repository: CBIG-02-006 DNA Extraction from Saliva*.

3.1 Reference to Other C-BIG SOPs or Documents

1. C-BIG Repository: CBIG-03-002 RPM Conversion
2. C-BIG Repository: CBIG-02-007 DNA Quantification Through NanoDrop Spectrophotometry

3.2 Reference to External SOPs or Documents

1. Experimental Therapeutic Program: SOP ETP-P-0003 DNA Extraction from Whole Blood
2. DNA Genotek Protocol: Oragene/saliva sample purification using the Puregene DNA purification kit

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who extract DNA from saliva. 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
EtOH	Ethanol
hr	Hours
mg	Milligram
mL	Milliliters
Min	Minutes
QA	Quality Assurance
QC	Quality Control
RT	Room Temperature
sec	Seconds
SOP	Standard Operating Procedure
˚C	Celsius
µL	Microliters

6. MATERIALS AND EQUIPMENT

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

NOTE: All sample contact materials must be suitable for RNA work (i.e., RNAse-free). Use clean gloves at all times to prevent inadvertent RNAse contamination during processing. All equipment, disposables or reagents can be substituted with equivalent materials following evaluation and approval, unless specified otherwise.

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.

Material/Equipment	Material/Equipment (site specific)
DNA saliva collection kit	DNA Genotek; Cat # OG-500
Cryovials, 1 mL, conical bottom	Nunc Biobanking and Cell Culture Cryogenic Tubes, Thermo Scientific; Cat # 366656
Disposable serological pipets 2 mL	
Disposable serological pipets 5 mL	
Disposable serological pipets 10 mL	
Polypropylene conical tubes 50 mL	
Pipets tips 200 µL	
Pipets tips 1000 µL	
Water-Bath	

Vortex	
Shaker	
Micropipet, range 100 µL to 1000 µL	
Clean workstation	
Pipet-Aid	
RNAse Solution A	Qiagen; Cat # 158924
Cell Lysis Solution	Qiagen, Cat # 158908
Protein Precipitation Solution	Qiagen, Cat # 158912
DNA Hydration Solution	Qiagen, Cat # 158916
Centrifuge with swinging bucket	Eppendorf centrifuge 5810, Cat # 022625004; with rotor S-4-104, Cat # 5820759003
-80°C Freezer	

7. PROCEDURES

PRECAUTIONS: All biological samples derived from a 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 under hospital guidelines.

NOTE: The saliva sample in the Oragene-DNA tube can be kept at RT indefinitely.

7.1 Extraction of human DNA from whole saliva

1. Incubate the Oragene/saliva sample at 50°C in a water-bath for one (1) hour.

2. Transfer the sample (4 mL) into a 50 mL polypropylene conical tube.
3. Add 1ml of Cell Lysis Solution and 25 µL of RNAse Solution A (4 mg/mL), vortex at high speed for 10 sec to mix sample and incubate 10 min at RT.
4. Add 1.67 mL of Protein Precipitation Solution to the cell lysate.
5. Vortex vigorously at high speed for 20 sec to mix uniformly.
6. Incubate the samples on ice for 5 min.
7. Centrifuge for 5 min at 2000xg using centrifuge with swinging bucket. If the pellet is not tight, repeat step 4 followed by incubation on ice for 5 min and then repeat step 5.
8. Pour the supernatant into 50 mL conical tube containing 5 mL of 2-propanol.
9. Mix tube by inverting gently 50 times.
10. Centrifuge for 3 min at 2000xg.
11. Carefully discard the supernatant, taking care that the pellet remains in the tube.
12. Wash the pellet with 5 mL 70% EtOH and invert tube several times to wash the DNA pellet.
13. Centrifuge for 1 min at 2000xg.
14. Discard supernatant.
15. Air dry the pellet for 5 to 10 min.
16. Add 400 µL DNA Hydration Solution to the pellet and rehydrate overnight at RT.
17. The next day if not ready to aliquot and quantify store the DNA at 4°C.

18. When aliquoted, store the DNA in a -80°C freezer

To aliquot and store the DNA please refer to the SOP CBIG-02-007 DNA quantification through NanoDrop Spectrophotometry.

7.2 Summary table

Steps
1. Incubate at 50°C for 1hr
2. Transfer into new 50 mL polypropylene conical tubes
3. Add 1 mL of Cell Lysis Solution
4. Add 25 µL of RNAse Solution A
5. Vortex for 10 sec
6. Add 1.67 mL Protein Precipitation Solution
7. Vortex for 20 sec
8. Incubate on ice for 5 min
9. Centrifuge for 5 min at 2000xg
10. Pour supernatant into a new tube containing 5 mL 2-propanol
11. Mix by gently inverting tube 50 times
12. Centrifuge for 3 min at 2000xg
13. Discard the supernatant
14. Add 5 mL of 70% EtOH
15. Centrifuge for 1 min at 2000xg
16. Discard the supernatant
17. Air-dry the pellet for 5 to 10 min
18. Add 400 µL DNA Hydration Solution
19. Store the DNA at RT overnight
20. Store the DNA at 4°C the next day.
21. Store in a -80°C freezer for long term storage

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/FORMS

9.1 Appendix A – Sample processing form: Saliva

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