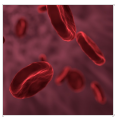

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DNA extraction from whole blood using simple salting out procedure

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bbzqip5w

Neurodegeneration Method Development Community

ABSTRACT

The purpose of this protocol is high-molecular weight DNA extraction from whole blood for genetic analyses, including no amplification long-range sequencing.

S. A. Miller, D. D. Dykes, H. F. Polesky (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research.
http://10.1093/nar/16.3.1215

ATTACHMENTS

DNA extraction from whole blood using simple salting out procedure.pdf

GUIDELINES

Storage Vial labeling:

3 lines, 6pt bold font; 1.5" label
a) Subject ID#: four digit SUSL ID (e.g. SUSL-2345), Sample type: *WBC*
b) Date of blood draw: MM/DD/YY
c) Study: [Name Study], Initials

MATERIALS

NAME	CATALOG #	VENDOR
Sodium chloride	S3014	Sigma Aldrich
Ammonium chloride (≥ 99.5 %)	A9434	Sigma Aldrich
Potassium bicarbonate (≥ 99.5 %)	90339	Sigma Aldrich
EDTA 500 mM Solution pH 8.0 ULTROL® Grade	324504	Merck Millipore
Trizma® hydrochloride / Tris-HCl	T5941	Merck Millipore Sigma
Ethyl Alcohol 200 Proof (GR ACS)	EX0276	Millipore Sigma
2-Propanol (99.5 %)	278475	Millipore Sigma
Proteinase K from Tritirachium album	P2308	Millipore Sigma

MATERIALS TEXT

If using different vendors, choose molecular biology grade reagents.

Buffers and Solution Recipes

10X Red blood cell (RBC) lysis buffer (1l)

NH4Cl	82.91 g
KHCO3	10.01 g
EDTA 0.5 M	20 ml
complete to 1000 ml with autoclaved dH2O	

* dilute to 1x before use with autoclaved dH_2O

1 M Tris-HCl, pH 8 (used to make nucleolysis buffer and TE)

Tris -HCl	15.7 g
Bring volume to ~ 95ml with autoclaved ddH2O	
measure & adjust pH to 8	
Bring final volume up to 100 ml with autoclaved ddH2O	

10X Nucleus lysis buffer (100ml)

NaCl	23.38 g
Tris-HCl (1 M, pH = 8)	10 ml
EDTA 0.5 M	4 ml
complete to 100 ml with autoclaved ddH2O	

* dilute to 1x before use with autoclaved ddH_2O

20 % SDS (used end of Day 1 for DNA extraction to poke holes in cell membrane)

SDS	20 g
autoclaved ddH2O	80 ml

5 M NaCl

NaCl	146.1 g
Bring volume to 500 ml with autoclaved dH2O	

70 % EtOH (used Day 2 of DNA extraction to “clean” DNA)

100 % EtOH	35 ml
ddH2O	15 ml

TE (used at end of DNA extraction to re-suspend DNA and prepare for storage)

1 M Tris-HCl	1 ml
0.5 M EDTA	0.2 ml
Bring volume to 100 ml with autoclaved ddH2O	

Proteinase K reconstitution:

Solutions can be prepared in [M]**25 Milimolar (mM) Tris-HCl buffer** , pH**8.0** , containing

[M]**1 Milimolar (mM) Calcium chloride** . Amounts for typical usage is [M]**50 µg/ml** – [M]**200 µg/ml** . Solutions are stable at pH**8**

at **4 °C** . It is stable in a broad range of environments: pH, buffer salts, detergents (SDS), and temperature. In the presence of 0.1 – 0.5 % SDS, proteinase K retains activity and will digest a variety of proteins and nucleases in DNA preparations without compromising the integrity of the isolated DNA.

Materials

- 10 ml EDTA collection tube (K_2EDTA, 5.4mg; 16 x 100mm; 10mL; Pink)
- 50 ml conical tube (Fisher catalog # 12565268)
- 1.5 ml clear Eppendorf tube (Fisher catalog # 5408129)
- 1000 µl filter pipette tips
- 5.0 ml serological pipette (Fisher catalog # 13-678-11D)
- 50 ml serological pipette (Fisher catalog # 13-678-11F)
- Gloves
- Bleach (e.g. Clorox, as 20 % fresh solution)

Equipment

- Centrifuge: Sorvall Legend XTR, or SorvallT 6000B, H10000B
- Centrifuge: Eppendorf 5417R
- Rotator

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

Prepare buffers and solutions (for recipes see 'Materials').

Red blood cell lysis

1 If you use FROZEN blood, start with step-case 'FROZEN blood'. If FRESH blood is used, select step-case 'FRESH blood'.

step case

FROZEN blood

2 In the morning take out tubes of blood from the **-80 °C** freezer. Record ID numbers and put in fridge (**4 °C**) to thaw until later in the afternoon.

3 Once blood has thawed, invert several times or place on rotator for a couple of minutes.

4 Decant the blood sample into 50 ml conical tube.

5 Rinse blood tube with **1 ml** of 1x RBC lysis buffer and add to 50ml conincal tube.

6 

Add *1x RBC lysis buffer* up to **50 ml** .

7 Place on shaker at **Room temperature** for **00:30:00** .

Note: Blood solution becomes transparent.

8 

Centrifuge **00:15:00** at **2000 rpm** at **Room temperature** .

9 Discard supernatant in container with **20 % bleach** .

Note: The pellet contains the nuclei.

If direct processing with Nuclei lysis, day 1, then continue **after this step** with Nuclei lysis **(step 12)**.

10 

Add **700 µl 1x RBC lysis buffer** and close the tube tightly. Pipette up and down to resuspend the pellet (cleans up the nuclei pellet from additional RBCs and allows for storage in small tubes).

11 Transfer to 1.5 ml **pre-labeled** clear Eppendorf tube.

12 

Centrifuge **00:05:00** at **2000 rpm** at **Room temperature** .

13 Using a 1000 µl pipette tip, discard the supernatant in container with **20 % bleach** .

14 Pellets can be frozen at this point at **-80 °C** .

Nuclei lysis (Day 1, afternoon)

15 

Add **3 ml Nucleus Lysis Buffer** and close tube tightly.

16 

Pipette up and down to resuspend pellet.

17 

Add **300 µl** of **10 % SDS** and **70 µl Proteinase K** (**10 mg/ml**).

18 

Mix by gently swirling tube.

19 

Incubate **Overnight** in water bath at **55 °C** .

DNA extraction (Day 2, morning)

20 

Add **1 ml** of **5 Molarity (M) NaCl** to the tubes.

21 Close caps tightly and **shake vigorously**.

Note: Very important to **shake thoroughly** at this step to precipitate proteins.

22 

Centrifuge for **00:20:00** at **3000 rpm** at **Room temperature** .

23 While centrifuging, label 2x 15ml conical tubes per sample.

24 Transfer supernatant to a 15 ml conical tube and then repeat centrifugation for **00:20:00** at **3000 rpm**

Note: The supernatant contains the DNA.

25 Transfer supernatant to next 15 ml conical tube.

26 

Add equal volume of **ice-cold** *isopropanol* (usually about **4 ml**).

27 Invert tubes several times and DNA will precipitate.

Note: You should be able to see **white strings** (this is the DNA!).

28 

Centrifuge for **00:05:00** at **3000 rpm** .

29 Discard supernatant.

30 

Add **1 ml** **70 % ethanol** to each tube.

31 

Centrifuge for **00:05:00** at **3000 rpm** .

32 

Carefully pipette off *ethanol* to not dislodge the DNA pellet.

33 Leave DNA to dry in **uncapped tubes** overnight.

Dissolve DNA

34 

Add **250 µl TE** and allow DNA to dissolve in 15 ml conical tube stored in fridge (**4 °C**).

35 Once **completely dissolved**, transfer to labeled sterile Eppendorf tube.

36 Label top with **subject ID**. Label side with **date of extraction** and **concentration**.

37 

Spec all samples using **Nanodrop**. Dilute samples to stock at **250 ng/µl** – **350 ng/µl** and to working dilution at **10 ng/µl** .

38 

Transfer samples into barcode tubes and log into DNA bank.

Red blood cell lysis

step case

FRESH blood

2 Decant the blood sample into 50 ml conical tube.

3 Rinse blood tube with **1 ml** 1xRBC lysis buffer and add to 50ml conical tube.

4 

Add *1x RBC lysis buffer* up to **50 ml** .

5 Place on shaker at **Room temperature** for **00:30:00** .

Note: Blood solution becomes transparent.

6 

Centrifuge **00:15:00** at **2000 rpm** at **Room temperature** .

7 Discard supernatant in container with **20 % bleach** .

Note: The pellet contains the nuclei.

If you continue with processing "Nuclei lysis, day 1", then continue **after this step** with Nuclei lysis **(step 13) and leave pellet in 50ml conical tube**.

8 

Add **700 µl 1x RBC lysis buffer** and close the tube tightly. Pipette up and down to resuspend the pellet (cleans up the nuclei pellet from additional RBCs and allows for storage in small tubes).

9 Transfer to 1.5 ml **pre-labeled** clear Eppendorf tube.

10 

Centrifuge **00:05:00** at **2000 rpm** at **Room temperature** .

11 

Using a 1000 µl pipette tip, discard the supernatant in container with **20 % bleach** .

12 Pellets can be frozen at this point at **-80 °C** .

Nuclei lysis (Day 1, afternoon)

13 

Add **3 ml Nucleus Lysis Buffer** and close tube tightly.

14 

Pipette up and down to resuspend pellet.

15 

Add **300 µl** of **10 % SDS** and **70 µl Proteinase K** (**10 mg/ml** stock solution).

The total volume is about 3370 plus the volume of the pellet for the nuclei lysis.
If 70ul of 10mg/ml proteinase K is added, it results in a dilution of ~1:50, which will be a final proteinase K concentration of 200ug/ml.

16 Mix by gently swirling tube.

17 

Incubate **Overnight** in water bath at **55 °C** .

Temperature is at 55C as proteinase K is resistant to denaturation by heat and will continue to function as long as the temperature/concentration is not too high, wherease most mucleases are denatureated at this temparature.

DNA extraction (Day 2, morning)

18 Add **1 ml** of **5 Molarity (M) NaCl** to the tubes.

19 Close caps tightly and **shake vigorously**.

Note: Very important to **shake thoroughly** at this step to precipitate proteins.

20 

Centrifuge for **00:20:00** at **3000 rpm** at **Room temperature** .

21 While centrifuging, label 2x 15ml conical tubes per sample.

22 

Transfer supernatant to a 15 ml conical tube and then repeat centrifugation for **00:20:00** at **3000 rpm**

Note: The supernatant contains the DNA.

23 

Transfer supernatant to next 15 ml conical tube.

24 

Add equal volume of **ice-cold** *isopropanol* (usually about **4 ml**).

25 Invert tubes several times and DNA will precipitate.

Note: You should be able to see **white strings** (this is the DNA!).

DNA after addition of 5M NaCl and vigorous shaking.

26 

Centrifuge for **00:05:00** at **3000 rpm** .

27 Discard supernatant.

28 

Add **1 ml** **[M]70 % ethanol** to each tube.

29 

Centrifuge for **00:05:00** at **3000 rpm** .

30 

Carefully pipette off *ethanol* to not dislodge the DNA pellet.

31 Leave DNA to dry in **uncapped tubes** overnight.

Dissolve DNA

32 

Add **100 µl** to **250 µl TE** and allow DNA to dissolve in 15 ml conical tube stored in fridge (**4 °C**).

33 

Once **completely dissolved**, transfer to labeled sterile Eppendorf tube.

34 Label top with **subject ID**. Label side with **date of extraction** and **concentration**.

35 

Spec all samples using **Nanodrop**. Dilute samples to stock at **[M]250 ng/µl** – **[M]350 ng/µl** and to working dilution at **[M]10 ng/µl** .

36 

Transfer samples into barcode tubes and log into DNA bank.