Aug 12, 2025

DNA Isolation from EDTA-blood

Brain

DOI
https://dx.doi.org/10.17504/protocols.io.14egnr2q6l5d/v1

Ann-Kathrin Hauser[1], Claudia Schulte[1]

[1]AG Gasser, Hertie Inst. for Clinical Brain Research, University of Tübingen.

Ann-Kathrin Hauser: Neurobiobank of The Hertie Inst. for Clinical Brain Research
Claudia Schulte: Neurobiobank of The Hertie Inst. for Clinical Brain Research

Julia Fitzgerald
Hertie Institute for Clinical Brain Research

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.14egnr2q6l5d/v1

External link: https://doi.org/10.1093/brain/awag135

Protocol Citation: Ann-Kathrin Hauser, Claudia Schulte 2025. DNA Isolation from EDTA-blood. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egnr2q6l5d/v1

Manuscript citation:
Anne Grünewald, Felix Knab, Milan Zimmermann, Zied Landoulsi, Jenny Ghelfi, Soraya Hezzaz, Sylvie Delcambre, Maria Zarani, Radu-Gabriel Copie, Benjamin Röben, Karan Sharma, Piero Parchi, Anna Katharina von Thaler, Ulrike Sünkel, Claudia Schulte, Ann-Kathrin Hauser, Christine Klein, Thomas Gasser, Bernd Wissinger, Kathrin Brockmann, Patrick May, Gerrit Machetanz, Julia C Fitzgerald (2026) Blood mtDNA markers of mitochondrial subtype and early-onset Parkinson's disease biology.Brain doi: 10.1093/brain/awag135

Protocol status: Working
We use this protocol and it's working

Created: August 12, 2025

Last Modified: August 12, 2025

Protocol Integer ID: 224527

Keywords: dna isolation from edta, dna isolation, edta vial, total dna for long term storage, erythrocyte, total dna, edta, blood whole blood, whole blood, blood

Abstract

Whole blood collected in EDTA vials (9-10mL) is processed to remove the erythrocytes before salting out the total DNA for long term storage at 4 deg C.

Guidelines

All research must adhere to the current, updated Helsinki declaration.
Prior ethical approval must be granted.
Informed consent must be given.

Materials

Erylysis-Buffer

NH_4Cl (molecular weight 53.49)	41.45g
$KHCO_3$ (molecular weight 100.12)	5g
EDTA-solution (250mM, pH 7.4)	2000µl
H_2Od	up to 5L

Store at 4°C

SE-Buffer

NaCl (molecular weight 58.44)	2.19g
EDTA-solution (250mM, pH 8)	50mL
H_2Od	up to 500mL

Store at room temperature

SDS-solution (10%)

Autoclave, store at room temperature

TE Buffer (10x)

TRIS (molecular weight 121.1)	6.057g
EDTA-solution (250mM. pH 7.4)	20mL
Adjust pH to 7.4	
H_2Od	Up to 500ml

Autoclave, and store at room temperature

Protocol materials

S-Monovette®, K3 EDTA **Sarstedt** **Catalog #**02.1066.001

Falcon tube 50 ml **Thermo Fisher Scientific** **Catalog #**1443222

Safety warnings

This protocol involves the processing of donated blood samples from routine hospital visits.
Ethical approval must be given by an IACUC or equivalent ethics committee prior to blood draw and processing.
Consent must be given by donors.

Ethics statement

The ethics committee of the Medical Faculty of the University of Tübingen and the University Clinic Tübingen (199/2011BO1 and 90/2009).

Before start

Ethical approvals in place.
Consent given.
Safety standards met for personnel to work with human blood.
Safety standards in place for disposal of human blood.

Removing the erythrocytes

1 Transfer whole blood from 9 mL S-Monovette®, K3 EDTA **Sarstedt Catalog #**02.1066.001 vial into 50 mL Falcon Falcon tube 50 ml **Thermo Fisher Scientific Catalog #**1443222 and fill with Erylysis buffer (see materials tab) up to 50 mL volume.

2 Store for 10 minutes at -20 °C

3 1700 rpm, 4°C Centrifuge 10 minutes (1700rpm or 600g)

Equipment

Centrifuge 5810 R	NAME
Refrigerated centrifuge	TYPE
Eppendorf	BRAND
5811000065	SKU
https://www.eppendorf.com/gb-en/eShop-Products/Centrifugation/Multipurpose-Centrifuges/Centrifuge-5810-5810R-p-PF-240994	LINK

4 .
Discard supernatant (in special waste container)

Safety information

All blood product waste should be treated according to local guidelines.

5 Fill the Falcon tube again with Erylysis-Buffer up to 30 mL and shake until the cell pellet
has dissolved.

6 Store for 10 minutes at -20 °C

7 Centrifuge 10 minutes at 4°C at 1700rpm (600g) in The Eppendorf 5810R centrifuge.

8 Discard supernatant (in sink) and put the Falcon tube containing the cell pellet upside down onto a tissue (to dry).

Disruption of the cell pellet

9 Add 5 mL SE-Buffer (see materials tab) and vortex vigorously until pellet has dissolved

10 Add 500 µL 10% SDS-Buffer and 5 µL Proteinase K (20mg/ml) and vortex again.

11 Store tube at 37 °C overnight or at Room temperature for 3 days.

Salting out

12 Add 2.5ml NaCl (5M) and vortex until it foams.

13 Centrifuge 10 minutes at 4°C at 2700rpm in The Eppendorf 5810R

14 If pellet is sharply defined, transfer supernatant into a new 50 mL
Falcon tube, that has been previously filled with 15 mL 100% Ethanol.
(If pellet is not sharply seen, then add 500 µL NaCL (5M), vortex again and centrifuge
again).

15 Rotate tube gently overhead to see DNA precipitating.

16 Fish the DNA to the side and discard the Ethanol.

17 Add 15 mL 70% Ethanol and gently shake.

18 Fish the DNA to the side and discard the Ethanol.

19 Add 15 mL 70%Ethanol and gently shake.

20 Transfer DNA to Cryotube (with pipette tip) and let it dry at room temperature.

21 Add 400 µL TE-Buffer (1X, see Materials tab) and shake at room temperature for at least 30 minutes.

Storage

22 Measure DNA

23 Store at 4 °C