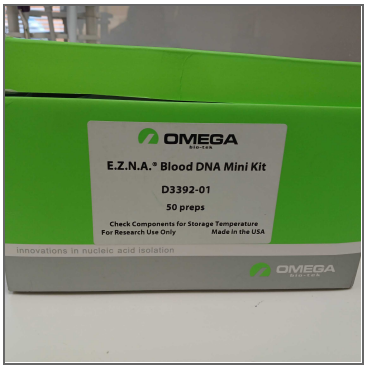

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External link:
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DNA extraction protocol for animal blood samples using the E.Z.N.A blood mini kit

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Biological clock measures the association between the circadian and epigenetic clock as predictors of migration and age

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ABSTRACT

The E.Z.N.A. Blood DNA Mini Kit provides an easy and rapid method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 250 µL fresh, frozen, or anticoagulated whole blood can be readily processed at one time. The E.Z.N.A. Blood DNA Mini Kit can also be used for the preparation of genomic DNA from buffy coat, serum, plasma, saliva, buccal swabs, and other body fluids. The E.Z.N.A. Blood DNA Kit allows for single or multiple simultaneous processing of multiple samples. There is no need for phenol/chloroform extractions, and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA obtained with the E.Z.N.A. Blood DNA Kit is ready for applications such as PCR, restriction digestion, and Southern blotting.

IMAGE ATTRIBUTION

https://www.omegabiotek.com/product/e-z-n-a-blood-dna-mini-kit/

GUIDELINES

Read and review the included product manual before starting.

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

Created: Jun 24, 2021

Last Modified: Nov 23, 2023

PROTOCOL integer ID: 51045

Keywords: DNA Extraction, Omega, Manual, Blood, Columns, Animals

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MATERIALS

- E.Z.N.A. Blood DNA kit **Whitehead Scientific Catalog #D3392-01**
- RNase I - 25,000 units **New England Biolabs Catalog #M0243L**
- 100% Ethanol **Contributed by users**
- Isopropanol **Contributed by users**
- Tabletop microcentrifuge capable of at least 13,000 x g
- Nuclease-free 2 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 65°C
- Vortexer

PROTOCOL MATERIALS

Isopropanol	Materials
E.Z.N.A. Blood DNA kit **Whitehead Scientific Catalog #D3392-01**	Materials
RNase I - 25,000 units **New England Biolabs Catalog #M0243L**	Materials, Step 2
100% Ethanol	Materials, Step 5

SAFETY WARNINGS

None

ETHICS STATEMENT

Protocol approval for the present study was obtained from the protocol committee of the Department of Genetics, University of the Free State (approval number: Res18/2020). Ethics approvals were obtained from the University of the Free State (approval number: UFS-AED2020/0015/1709) as well as the South African National Biodiversity Institute (approval number: SANBI/RES/P2020/30). Appropriate research permits were also obtained from South African regulatory authorities including the Department of Agriculture, Land Reform, and Rural Development (Section 20 permit: 12/11/1/1/18(1824JD)) and the Department of Environmental Affairs (Threatened Or Protected Species (TOPS) permit: O-52903).

BEFORE START INSTRUCTIONS

- Prepare HBC Buffer and DNA Wash Buffer according to the directions.
- Set water bath, incubator, or heat block to 65°C.
- Heat the Elution Buffer to 65°C.

Lyse

1 Transfer the Sample into a sterile microcentrifuge tube and bring the volume up to 250 µL with 10mMTris-HCl, PBS, or Elution Buffer (provided).

2 Add 25 µL Proteinase K Solution (provided), 250 µL BL Buffer (provided), and 5 µL RNase I - 25,000 units **Whitehead Scientific Catalog #M0243L** (50 mg/mL).

3 Vortex at maximum speed for 00:00:15 seconds. 15s

4 Incubate at 65 °C for 00:10:00 minutes. Vortex briefly once during incubation. 10m

Heating block set to 65 degrees Celsius.

Adjust binding conditions

5 Add 260 µL 100% Ethanol Whitehead Scientific. Vortex at maximum speed for 00:00:20 (20s) seconds.

6 Centrifuge briefly to collect any drops from the inside of the lid.

Bind

7 Insert a HiBind DNA Mini Column (provided) into a 2 mL Collection Tube.

Green HiBind columns to be placed in clear collection tubes. Can label samples on cap.

8 Transfer the entire sample to the column.

9 Centrifuge at 11000 x g, 21°C, 00:01:00 1m

10 Discard the filtrate and the Collection Tube. Insert the HiBind DNA Mini Column into a new 2 mL Collection Tube.

11 Add 500 µL HBC Buffer.

Note

HBC Buffer must be diluted with 100% isopropanol before use.

12 Centrifuge at 11000 x g, 21°C, 00:01:00 1m

13 Discard the filtrate and reuse Collection Tube.

Wash

14 Add 700 µL DNA Wash Buffer (provided).

Note

DNA Wash Buffer must be diluted with 100% ethanol before use.

15 Centrifuge at 10000 x g, 21°C, 00:01:00 1m

16 Discard the filtrate and reuse the Collection Tube.

17 go to step #14 Repeat once

Dry

18 Centrifuge the empty HiBind DNA Mini Column at 13000 x g, 21°C, 00:02:00 . 2m

Note

It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

Elute 7m

19 Transfer the HiBind DNA Mini Column into a nuclease-free 2 mL microcentrifuge tube.

20 Add 50 µL Elution Buffer heated to 65 °C .

21 Incubate the HiBind DNA Mini Column at 65 °C for 00:05:00 . 5m

22 Centrifuge at 13000 x g, 21°C, 00:02:00 . 2m

23 **Optional:** Apply filtrate to column and repeat centrifugation.

*

Store

24 Store eluted DNA at -20 °C .

Samples extracted using this protocol were submitted to NCBI BioSample and linked to BioProject PRJNA737185.

Expected result

Nanodrop One results for samples:

	Sample	A260	A280	A260/A230	A260/A280	ng/uL
	1	0.185	0.096	0.359	1.934	9.26
	2	0.125	0.059	0.732	2.131	6.26
	3	0.159	0.102	0.528	1.558	7.94
	4	0.082	0.047	0.405	1.723	4.08
	5	0.107	0.058	0.815	1.838	5.33
	6	0.110	0.063	1.020	1.742	5.51
	7	0.143	0.070	0.518	2.033	7.14
	8	0.077	0.029	1.443	2.615	3.83
	9	0.060	0.039	1.156	1.534	2.99
	10	0.059	0.032	0.398	1.844	2.95
	11	0.082	0.044	1.039	1.855	4.10
	12	0.119	0.073	1.025	1.637	5.96
	13	0.072	0.032	0.938	2.237	3.60
	14	0.098	0.051	1.051	1.918	4.88
	15	0.092	0.049	1.475	1.869	4.59

The extractions yielded pure DNA (A260/A280 approximately 1.6 to 1.8) with sufficiently high concentrations of around 4 ng/uL.