DNAbler – Blood 2.0

DNA extraction kit from whole blood, serum/plasma, liquid clinical samples, buccal swabs, and mammalian tissue using spin-column based technology

Catalog Number:

DE26010

DE26050

Product Manual (Version 1.3)

This product is shipped and stored at room temperature. For long term storage, Proteinase K and RNase A should be stored at-20°C.

This product is intended for research use only (RUO). It is not intended for use in medical diagnosis.

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A. Kit overview

Specifications and intended use

This kit is intended for extraction of high-quality DNA from blood. The procedure is quick and simple, and can be adapted to high-throughput applications. Moreover, cell-free DNA can be quickly isolated from serum or plasma. An experienced user can extract DNA from up to 16 samples in less than an hour.

Our protocols are compatible with freshly drawn or frozen whole blood or serum. Do not use blood supplemented with Heparin, as heparin is known to inhibit PCR.

THIS KIT SHOULD **NOT** BE USED FOR MEDICAL OR DIAGNOSTIC PURPOSES.

Safety precautions

The Lysis/Binding and Wash1 buffers contain a guanidine salt, which is a known irritant that is harmful if inhaled, swallowed, or came in contact with skin. Wear appropriate protective equipment and be extremely cautious when handling and discarding the Lysis/Binding Buffer.

Do **NOT** mix Lysis/Binding or Wash1 buffers with bleach.

Refer to the product's webpage for full SDS sheets.

Before first use

It is imperative to add 96-100% ethanol to Wash1 and Wash2 Buffer before first use:

Component	DE26010	DE26050
Ethanol Added to Wash1	3.3 mL	16.5 mL
Ethanol Added to Wash2	9.6 mL	48 mL

Components

Component	DE26010 (10 extractions)	DE26050 (50 extractions)
Lysis/Binding Buffer	6 mL	30 mL
Wash1 Buffer (after ethanol addition)	6 mL	30 mL
Wash2 Buffer (after ethanol addition)	12 mL	60 mL
Elution Buffer	1 mL	5 mL
Spin columns*	10	50
Proteinase K	0.2mL	1mL
RNase A	0.2mL	1mL

* nucleic acid binding capacity ~50µg

Additionally, you will need the following items that are not supplied with the kit:

- Thermal shaker capable of reaching 60°C with 1.5mL tube block
- 1.5mL DNase/RNase-free tube per sample for sample processing (2.0mL for plasma samples)
- 1.5mL DNase/RNase-free tube per sample for elution
- Centrifuge with rotor for 1.5mL tubes capable of producing at least 10,000g
- Vortex
- Pipettes and DNase/RNase-free tips
- 96-100% ethanol
- PBS (for tissue or buccal swabs)
- For tissue samples: rotor-stator homogenizer OR bead-beater homogenizer OR mortar and pestle with liquid nitrogen

Storage and stability

This kit is shipped at room temperature. Store all components in a dry place at room temperature. For longer storage, Proteinase K and RNase A should be stored at -20°C. All components are stable for at least a year from the indicated production date.

RNase treatment

Inclusion of RNase in this kit ensures purification of pure DNA with minimal, if any, RNA contamination. Our internal testing shows that, when RNase is used, the qPCR signal of contaminating RNA can be reduced ≥ 100-fold.

Our RNase is treated to remove any possible DNase contamination, and our tests reveal absence of any DNase activity.

B.Protocols for DNA extraction

Whole blood

In our experience, using ice cold ethanol (-20°C) in the binding step (step6 in this protocol) usually gives better purity in older, frozen blood samples.

Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature. ***Do NOT premix the proteinase K and RNase A.***

1. Switch on the thermal shaker and set it on 55-60°C
2. Gently invert the blood tube upside down several times, then transfer 200µL of whole blood from each blood sample to a separate clean, sterile 1.5mL tube
3. Add 200µL of Lysis/Binding Buffer and 20µL of proteinase K, vortex briefly and spin down, then incubate at 55-60°C for 10-20 minutes, the lysate should turn black upon addition of the Lysis/Binding Buffer
4. Set the lysate-containing tube aside at room temperature for 2 minutes, spin down to collect droplets that have condensed on the inside of the lid
5. (Optional RNase A Treatment) Add 20µL of RNase A, vortex briefly and spin down, then incubate at room temperature for 5 minutes
6. Add 200µL of 96-100% ethanol, vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)
7. Apply the whole lysate-ethanol mixture to a spin column embedded in a collection tube, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

   ➔ ***you may observe some black discoloration on the spin column filter but that is fine***

8. Add 500µL of Wash1 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

9. Add 500µL of Wash2 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

10. Add 500µL of Wash2 Buffer to the spin column, centrifuge at 12,000g for 2 minutes, discard the collection tube and proceed to the next step

11. Place the spin column in a new, DNase/RNase-free 1.5mL tube (elution tube), add 30-100µL of Elution Buffer directly to the center of the spin column filter without touching the filter with the tip, incubate for 2 minutes at room temperature

Hint: pre-heating the Elution Buffer at 60-70°C may increase the yield

12. Centrifuge, discard the spin column, the DNA-containing eluate is ready for use in downstream analysis (use immediately or place at -20°C for short term storage, -80°C for long term storage)

13. Measure the purity of your eluate with a spectrophotometer, pure DNA should have 260/280 ratio ~1.8 and 260/230 ratio ~2 – 2.2

Serum/plasma

Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature. ***Do NOT premix the proteinase K and RNase A.***

1. Switch on the thermal shaker and set it on 55-60°C

1. Transfer 400µL of serum/plasma to a separate clean, sterile 1.5mL tube

2. Add 400µL of Lysis/Binding Buffer and 20µL of proteinase K, vortex briefly and spin down, then incubate at 55-60°C for 10-20 minutes

3.

4. Set the lysate-containing tube aside at room temperature for 2 minutes, spin down to collect droplets that have condensed on the inside of the lid

5. (Optional RNase A Treatment) Add 20µL of RNase A, vortex briefly and spin down, then incubate at room temperature for 5 minutes

6. Add 800µL of 96-100% ethanol, vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)

7. Apply up to 700µL of the lysate-ethanol mixture to a spin column embedded in a collection tube, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

8. Apply the remaining lysate-ethanol mixture, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column

9. Add 500µL of Wash1 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

10. Add 500µL of Wash2 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

11. Add 500µL of Wash2 Buffer to the spin column, centrifuge at 12,000g for 2 minutes, discard the collection tube and proceed to the next step

12. Place the spin column in a new, DNase/RNase-free 1.5mL tube (elution tube), add 30-50µL of Elution Buffer directly to the center of the spin column filter without touching the filter with the tip, incubate at room temperature for 2 minutes

Hint: pre-heating the Elution Buffer at 60-70°C may increase the yield

13. Centrifuge, discard the spin column, the DNA-containing eluate is ready for use in downstream analysis (use immediately or place at -20°C for short term storage, -80°C for long term storage)

14. DNA concentration in serum/plasma is usually below the detection limit of most spectrophotometric techniques. We recommend assessing the DNA directly by using it in the downstream application (PCR, qPCR, library preparation)

Liquid clinical samples

This protocol is compatible with a wide range of liquid clinical samples including viral transport media (VTMs), and saliva. If you are using different sample types, please contact our technical support team.

Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature. ***Do NOT premix the proteinase K and RNase A.***

1. Switch on the thermal shaker and set it on 55-60°C

1. Transfer 200µL of the sample to a separate clean, sterile 1.5mL tube

2. Add 200µL of Lysis/Binding Buffer and 20µL of proteinase K, vortex briefly and spin down, then incubate at 55-60°C for 10-20 minutes (for saliva, increase incubation to 30-60 minutes, or until the sample is completely homogenized)

3. Set the lysate-containing tube aside at room temperature for 2 minutes, spin down to collect droplets that have condensed on the inside of the lid

4. (Optional RNase A Treatment) Add 20µL of RNase A, vortex briefly and spin down, then incubate at room temperature for 5 minutes

5. Add 400µL of 96-100% ethanol, vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)

6. Apply the whole lysate-ethanol mixture to a spin column embedded in a collection tube, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

7. Add 500µL of Wash1 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

8. Add 500µL of Wash2 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

9. Add 500µL of Wash2 Buffer to the spin column, centrifuge at 12,000g for 2 minutes, discard the collection tube and proceed to the next step

10. Place the spin column in a new, DNase/RNase-free 1.5mL tube (elution tube), add 30-50µL of Elution Buffer directly to the center of the spin column filter without touching the filter with the tip, incubate at room temperature for 2 minutes

Hint: pre-heating the Elution Buffer at 60-70°C may increase the yield

11. Centrifuge, discard the spin column, the eluate is ready for use in downstream analysis (use immediately or place at -20°C for short term storage, -80°C for long term storage)

12. DNA concentration in many clinical samples (e.g. cerebrospinal fluid, VTM, etc.) is usually below the detection limit of most spectrophotometric techniques. We recommend assessing the DNA directly by using it in the downstream application (PCR, qPCR, library preparation, etc.)

Buccal swabs

DNA yield depends on the number of cells collected while scraping the cheeks of the subject. To maximize the yield, make sure the subject does not eat or drink for at least 1 hour before sample collection, scrape the inside of each cheek firmly ~5 times, and dry the buccal swab at room temperature for at least 1 hour before extraction.

Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature. ***Do NOT premix the proteinase K and RNase A.***

1. Switch on the thermal shaker and set it on 55-60°C

1. Add 400µL of PBS to a 1.5mL tube, insert the buccal swab, and vortex thoroughly

2. Add 20µL of proteinase K, vortex briefly and spin down, then incubate at 55-60°C for 10-20 minutes

3. Set the lysate-containing tube aside at room temperature for 2 minutes, spin down to collect droplets that have condensed on the inside of the lid

4. (Optional RNase A Treatment) Add 20µL of RNase A, vortex briefly and spin down, then incubate at room temperature for 5 minutes

5. Add 400µL of Lysis/Binding Buffer, vortex briefly and spin down, add 400µL of 96-100% ethanol, vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)

6. Apply 700µL of the lysate-ethanol mixture to a spin column embedded in a collection tube, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

7. Apply the remaining lysate-ethanol mixture, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column

8. Add 500µL of Wash1 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

9. Add 500µL of Wash2 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

10. Add 500µL of Wash2 Buffer to the spin column, centrifuge at 12,000g for 2 minutes, discard the collection tube and proceed to the next step

11. Place the spin column in a new, DNase/RNase-free 1.5mL tube (elution tube), add 30-50µL of Elution Buffer directly to the center of the spin column filter without touching the filter with the tip, incubate at room temperature for 2 minutes

Hint: pre-heating the Elution Buffer at 60-70°C may increase the yield

12. Centrifuge, discard the spin column, the DNA-containing eluate is ready for use in downstream analysis (use immediately or place at -20°C for short term storage, -80°C for long term storage)

13. Measure the purity of your eluate with a spectrophotometer, pure DNA should have 260/280 ratio ~1.8 and 260/230 ratio ~2 – 2.2

Mammalian tissue

Use this protocol to extract DNA from up to 25mg of fresh tissue (10mg of spleen), tissue preserved in RNALater (or equivalent), or tissue preserved in -80°C.

Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature. ***Do NOT premix the proteinase K and RNase A.***

1. Switch on the thermal shaker and set it on 55-60°C

1. Lyse and homogenize the tissue by one the following methods:

    a) Cut the tissue to small pieces, add it to 200µL of PBS in a 1.5mL tube, and homogenize with a rotor stator homogenizer following the instrument's instructions; 15-30 seconds should be sufficient for complete lysis

    b) Add 200µL of PBS to a 1.5mL tube, cut the tissue to small pieces and add it to the tube, add 1-2mm sterile steel beads, homogenize with a bead beater following the manufacturer's instructions

    c) Place the tissue on a mortar, add liquid nitrogen, crush it thoroughly with a pestle into a fine powder, allow the liquid nitrogen to evaporate, quickly transfer the powder to a tube containing 200µL of PBS

    d) (optional for soft tissue, e.g. brain, kidney, etc. <5mg) cut the tissue to small pieces, add it to 200µL of PBS and homogenize with a manual sterile pestle that fits the bottom of your tube until no small pieces are visible

2. Add 20µL of proteinase K, vortex briefly and spin down, incubate at 55-60°C until the tissue is completely lysed (usually 1-2 hours)

    ➔ ***For more difficult samples (e.g. rat tails) increase incubation times to up to 10 hours or until tissue is completely lysed***

3. (optional RNase-treatment) Add 20µL of RNase A, vortex briefly and spin down, incubate at room temperature for 5 minutes

4. Add 200µL of Lysis/Binding Buffer, vortex briefly and spin down

➔ ***If the lysate contains excess cell debris, pellet the debris by centrifugation at >14,000g for 5 minutes, then carefully transfer as much as possible of the supernatant to a clean DNase/RNase-free 1.5mL tube***

5. Add 200µL of 96-100% ethanol, vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)

6. Apply the whole lysate-ethanol mixture to a spin column embedded in a collection tube, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

7. Add 500µL of Wash1 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

8. Add 500µL of Wash2 Buffer to the spin column, centrifuge, discard the flow-through, dab the drop on the edge of the collection tube on a lint-free tissue until dry then replace the spin column in the collection tube

9. Add 500µL of Wash2 Buffer to the spin column, centrifuge at 12,000g for 2 minutes, discard the collection tube and proceed to the next step

10. Place the spin column in a new, DNase/RNase-free 1.5mL tube (elution tube), add 30-100µL of Elution Buffer directly to the center of the spin column filter without touching the filter with the tip, incubate at room temperature for 2 minutes

Hint: pre-heating the Elution Buffer at 60-70°C may increase the yield

11. Centrifuge, discard the spin column, the DNA-containing eluate is ready for use in downstream analysis (use immediately or place at -20°C for short term storage, -80°C for long term storage)

12. Measure the purity of your eluate with a spectrophotometer, pure DNA should have 260/280 ratio ~1.8 and 260/230 ratio ~2 – 2.2

C. Appendix

Troubleshooting

Problem	Possible reason	Solution
Spin column clogged	▪ Too much sample was used	▪ Pipetting the lysate in the clogged column up and down followed by another centrifugation at 15,000g for 3 minutes in the binding step may salvage your sample, but next time use less amount of sample
	▪ Proteinase K was not added	▪ Ensure addition of proteinase K as recommended in the protocol
	▪ Too much cell debris in the lysate	▪ Centrifuge lysate at 15,000g for 10 minutes, obtain supernatant and discard the pellet
Low DNA yield	▪ Little or no Ethanol added to lysate	▪ Make sure to add ethanol before binding
	▪ Ethanol was not added to Wash1 or Wash2 Buffer before use	▪ Make sure to add ethanol to Wash1 and Wash2 Buffer as described
	▪ Too much sample was used	▪ Use less amount of sample
Degraded DNA	▪ DNase contamination	▪ Use DNase/RNase-free plastic-ware
	▪ Improper handling or storage of the sample before lysis	▪ Avoid repeated freeze/thaw cycles, minimize the time of keeping the DNA in temperatures higher than -20°C

Quality control

All lots are tested by extracting DNA from 200µL of human blood, measuring concentration and purity by spectrophotometry (260/280 and 260/230 ratios), and visualization of the extracted DNA band size on a gel.

Support

For questions, suggestions, or technical support, feel free to contact us by email on:

support@havensci.com