DNAbler – Blood

DNA extraction kit from whole blood, serum/plasma, or liquid clinical samples using spin-column based technology

Catalog Numbers:

DE96010

DE96050

DE96250

Product Manual (Version 2.2)

This product is shipped in two boxes. Box1 components should be stored at room temperature. Box2 should be placed at -20°C immediately upon receipt.

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 (with or without EDTA). 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 Buffer contains 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 Buffer with bleach.

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

- **Before first use**

It is imperative to add 96-100% ethanol to Wash2 Buffer before first use, as follows:

DE96010 → 8 mL EtOH

DE96050 → 48 mL EtOH

DE96250 → 240 mL EtOH

- **Components**

		DE96010 (10 extractions)	DE96050 (50 extractions)	DE96250 (250 extractions)
Box 1	**Lysis/Binding Buffer**	10 mL	30 mL	150 mL
	Wash1 Buffer	10 mL	30 mL	150 mL
	Wash2 Buffer (after ethanol addition)	20 mL	60 mL	300 mL
	Elution Buffer	2 mL	5 mL	25 mL
	Spin columns*	10	50	250
Box 2	**Proteinase K**	0.2mL	1mL	5mL
	RNase A	0.2mL	1mL	5mL

* nucleic acid binding capacity ~50µg

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

- 1.5mL DNase/RNase-free tube per sample (elution tube)
- Centrifuge with rotor for 1.5mL tubes capable of producing at least 10,000g
- Vortex
- Pipettes and DNase/RNase-free tips
- 96-100% ethanol

- **Storage and stability**

This kit is shipped in two boxes. Store Box2 at -20°C immediately upon receipt. Store all other components of Box1 in a dry area at room temperature. All components of the kit 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**

- **<u>Whole blood</u>**

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

1. Switch on a water bath or a heat block 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. Follow step 3(a) to perform the optional RNase-treatment or step 3(b) if you wish to skip it

   a) Add 20µL of proteinase K, incubate at 55-60°C for 5 minutes, add 20µL of RNase A, vortex briefly and spin down, incubate at room temperature for 5 minutes
   b) Add 20µL of proteinase K

4. Add 200µL of Lysis/Binding Buffer, vortex briefly and spin down, then incubate at 55-60°C for 10 minutes with occasional vortexing, the lysate should turn black upon addition of the Lysis/Binding Buffer

5. Spin down the tube to collect droplets that have condensed on the inside of the lid then 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

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

7. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through

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. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through

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. Repeat steps 10-11 for a second washing step with Wash2 Buffer

12. Discard the collection tube and 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

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. 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 a water bath or a heat block and set it on 55-60°C

2. Transfer 300µL of serum/plasma to a separate clean, sterile 1.5mL tube

3. Follow step 3(a) to perform the optional RNase-treatment or step 3(b) if you wish to skip it

   c) Add 20µL of proteinase K, incubate at 55-60°C for 5 minutes, add 20µL of RNase A, vortex briefly and spin down, incubate at room temperature for 5 minutes
   d) Add 20µL of proteinase K

4. Add 300µL of Lysis/Binding Buffer, vortex briefly and spin down, then incubate at 55-60°C for 10 minutes with occasional vortexing

5. Spin down the tube to collect droplets that have condensed on the inside of the lid then add 600µL 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 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

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. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through

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. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through

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 you to assess 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 a different type of samples, 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 a water bath or a heat block and set it on 55-60°C

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

3. Follow step 3(a) to perform the optional RNase-treatment or step 3(b) if you wish to skip it

   e) Add 20µL of proteinase K, incubate at 55-60°C for 5 minutes, add 20µL of RNase A, vortex briefly and spin down, incubate at room temperature for 5 minutes
   f) Add 20µL of proteinase K

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

5. Spin down the tube to collect droplets that have condensed on the inside of the lid then 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. Centrifuge the spin column empty for 10 seconds then proceed to the next step without discarding the flow-through

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. Centrifuge the spin column empty 10 seconds then proceed to the next step without discarding the flow-through

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

C. **Appendix**

- **<u>Troubleshooting</u>**

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 Wash2 Buffer before use	▪ Make sure to add ethanol to 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 performing real-time PCR using DNA specific assays.

- **Support**

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

support@havensci.com