DNAbler – Cells and Tissue

Genomic DNA extraction kit from cells and tissue using spin-column based technology

Catalog Number:

DE25010

DE25050

Product Manual (Version 1.2)

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 quick extraction of high-quality DNA from animal cells and tissue, as well as bacteria. It is also possible to cleanup DNA samples with this kit, whether after a separate RNase treatment step or to remove contaminants.

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 Wash1 and Wash2 Buffer before first use:

Component	DE25010	DE25050
Ethanol Added to Wash1	3.3 mL	16.5 mL
Ethanol Added to Wash2	9.6 mL	48 mL

Components

Component	DE25050 (50 extractions)	DE25010 (10 extractions)
Digestion Buffer	20 mL	4 mL
Lysis/Binding Buffer	30 mL	6 mL
Wash1 Buffer (after ethanol addition)	30 mL	6 mL
Wash2 Buffer (after ethanol addition)	60 mL	12 mL
Elution Buffer	5 mL	1 mL
Spin columns*	50	10
Proteinase K	1mL	0.2mL
RNase A	1mL	0.2mL

* 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
- 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
- For tissue samples: rotor-stator homogenizer OR bead-beater homogenizer OR mortar and pestle with liquid nitrogen
- For adherent cells: PBS
- For gram-positive bacteria: lysozyme and lysozyme buffer (20 mM Tris-HCl, 2.5 mM EDTA, 1% Triton X-100)

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 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 ≥ 1,000-fold.

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

B. Protocols for DNA extraction

Mammalian tissue

Use this protocol to extract DNA from up to 25mg (10mg of spleen) of fresh tissue, 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

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

    a) Cut the tissue to small pieces, add it to 200µL of Digestion Buffer 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 Digestion Buffer 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 Digestion Buffer

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

3. 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***

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

5. Add 200µL of the 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***

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

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 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 cells

With this protocol DNA can be extract from 10^4-10^7 mammalian cells.

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. To digest cells, use step 2a for adherent cells and step 2b for suspension cells:

    a) Discard as much media as possible, rinse with PBS, gently scrape the cells and transfer them to a clean DNase/RNase-free 1.5mL tube, add 200µL of Digestion Buffer

    b) Pellet the cells by centrifugation at 1,000g for 10 minutes, discard the cell media (be careful not to disrupt the pellet), use a 10-20µL pipette to remove the remaining media from around the pellet, add 200µL of Digestion Buffer

2. Add 20µL of proteinase K, vortex briefly and spin down, incubate at 55-60°C until the cells are completely lysed (usually 10-20 minutes)

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

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

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

Bacteria

With this protocol DNA can be extracted from up to 10^9 bacterial cells.

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. To digest cells, use step 2a for gram negative and step 2b for gram positive bacteria:

    a. Pellet the gram-negative bacteria by centrifugation at 10,000g for 2 minutes, discard the cell media (be careful not to disrupt the pellet), use a 10-20µL pipette to remove the remaining media from around the pellet, add 200µL of Digestion Buffer

    b. Pellet the gram-positive bacteria by centrifugation at 10,000g for 2 minutes, discard the cell media (be careful not to disrupt the pellet), use a 10-20µL pipette to remove the remaining media from around the pellet, add 200µL of lysozyme buffer (20 mM Tris-HCl, 2.5 mM EDTA, 1% Triton X-100) and, immediately before use, add fresh lysozyme for final concentration of 20mg/mL, incubate at 37°C for 30 minutes

3. Add 20µL of proteinase K, vortex briefly and spin down, incubate at 55-60°C until the cells are completely lysed (usually 10-20 minutes)

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

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

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

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 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

DNA Cleanup

This is a quick, phenol-free protocol to cleanup your DNA samples after a separate RNase treatment or to remove impurities.

Unless otherwise stated, all centrifugation steps should be at 10,000g for 1 minute at room temperature.

1. Adjust the volume of your DNA sample to 100µL with Elution Buffer or RNase-free water

2. Add 250µL of Lysis/Binding Buffer, followed by 350µL of 96-100% ethanol, vortex for 30-60 seconds then spin down the tube for 1-2 seconds only (do not exceed 2 seconds)

3. Apply the whole 750µL of the DNA-Lysis Buffer-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

4. 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

5. 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

6. 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 improve the yield

7. 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)

8. 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	▪ Up and down pipetting and 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 animal tissue, 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