EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMAGNET Human and Animal Tissue DNA Purification Kit

Kit for isolation of total DNA from human and animal tissues

Cat. no. E3425

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Table of Contents

Content	96 preps E3425-01	Storage/Stability
Lyse T	10 ml	15-25°C
RNase A (10 mg/ml)	0.22 ml	2-8°C
Proteinase K (20 mg/ml)	2.2 ml	–20°C
Sol T	58 ml	15-25°C
Wash T1	46 ml	15-25°C
Wash T2	70 ml	15-25°C
Wash T3	70 ml	15-25°C
Elution	30 ml	15-25°C
Magnetic Beads	1 ml	2-8°C
Protocol	1	

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for isolation of total DNA (genomic, mitochondrial) from a variety of tissues and biological liquids by silica-covered Magnetic Beads. The kit is designed for manual or automatic use.

NOTE 2 · Maximum Sample Amount. One preparation enables purification of DNA from up to 25 mg solid tissues or 100 µl liquid tissues.

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature, with the exception of Magnetic Beads, RNase A and Proteinase K. Magnetic Beads and RNase A should be kept at 2-8°C and Proteinase K at -20°C.

NOTE 4 · Internal control. Commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control DNA should be added to the lysis buffer Lyse T.

NOTE 5 · Additional recommendations. Avoid cross contamination of samples during isolation procedure by changing pipette tips whenever necessary.

NOTE 6 · DNA purification efficiency. Working with Magnetic Beads, depending on the source material, the user can obtain high DNA concentrations of the isolates. PCR optimization is required in order to avoid nonspecific products or low reaction efficiency. The optimal genomic DNA template is 5-50 ng per 50 µl PCR reaction volume.

NOTE 7 · Maintaining Good Working Practice. All solutions should be kept tightly closed to avoid evaporation and resulting concentration changes of buffer components. To obtain high quality DNA, stick carefully to the protocol provided below.

NOTE 8 · Elution buffer is a low salt solution, that contains no metal ion chealators (e.g. EDTA) that can inhibit subsequent enzymatic reactions. Elution buffer composition is suitable for downstream applications such as digestion with restriction enzymes, phosphorylation, ligation, Sanger sequencing, NGS etc. It is also possible to elute the DNA with Tris-HCl, water or TE.

Equipment and reagents to be supplied by the user

- Magnetic stand E0361 for 16 tubes, E0362 for 24 tubes, E0363 for 96-well plate. To be purchased separately.
- Microcentrifuge, disposable gloves, sterile pipette tips, sterile 1.5-2 ml tubes or 96-well plates with working volume at least 800 µl, a heating block capable of incubation at 37-56°C.
- [1 M] Dithiothreitol (DTT), ethanol [96 -100% v/v], xylene, PBS (cat. no E0281) and 10 mM Tris pH 8.0 (add 10 ml 1M Tris-HCl pH 8.0 EURx cat. no E0273 to 990 ml sterile water).

Protocol

NOTE 1 • Tissue digestion with Proteinase K can be performed in the room temperature (56°C recommended) prolonging the incubation time.

Part I Sample preparation

A. Solid tissues/Insects/Rodent tails

1. Choose tissue homogenization method.

   a) Grind tissue fragment under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (up to 25 mg) in 2 ml Eppendorf tube and centrifuge the powder to the bottom of the tube. Add 200 µl of PBS and suspend the precipitate thoroughly.

      - *To obtain high yield of DNA a tissue fragment should be thoroughly grinded to a fine powder.*

   b) Place up to 25 mg of tissue in 2 ml Eppendorf tube. Add 200 µl PBS and homogenize the sample using a mechanical homogenizer.

   c) Cut tissue fragment (up to 25 mg) into small pieces. Place the sample in 2 ml Eppendorf tube and add 200 µl of PBS.

2. Add 2 µl of **RNase A**, 20 µl of **Proteinase K** and 80 µl **Lyse T**. Mix by inverting or vortexing the tube.

3. Incubate at 56°C until the tissue is completely lysed (at least 1-3 h). Mix by inverting or vortexing every 15-30 min.

   - *Samples can be lysed overnight, if needed.*

4. Spin down with maximal speed for 3 min and transfer the supernatant into the new tube.

   - *This step enables to remove undigested tissue fragments, hair and bones.*
   - *This step can be omitted if the sample is digested completely.*

5. Follow the point 1. Part II. of the DNA isolation protocol.

B. Paraffin-embedded tissues

1. Prepare a small section (up to 25 mg) from block of embedded tissue. Place the sample in 2 ml Eppendorf tube.

2. Add 1 ml xylene. Vortex vigorously. Incubate at room temperature for 15 min.

3. Centrifuge for 3 min at maximal speed. Remove supernatant by pipetting.

4. Add 1 ml xylene to the pellet, mix by vortexing.

5. Centrifuge for 3 min at maximal speed. Remove supernatant by pipetting.
6. Add 1 ml ethanol (96–100%) to the pellet. Mix by vortexing or inverting the tube.
7. Centrifuge for 3 min at maximum speed. Remove supernatant by pipetting.
8. Repeat steps 6–7 once.
9. Incubate the open tube at 37°C until the ethanol has evaporated (app. 15 min).
10. Resuspend the tissue pellet in 200 µl of PBS.
11. Add 2 µl of **RNase A**, 20 µl of **Proteinase K** and 80 µl **Lyse T** and 80 µl **Lyse T**. Mix by inverting or vortexing the tube.
12. Incubate at 56°C until the tissue is completely lysed (at least 1-3 h). Mix by inverting or vortexing every 15-30 min.
    - *Samples can be lysed overnight, if needed.*
13. Follow the point 1. Part II. of the DNA isolation protocol.

C. Formalin-fixed tissues

1. Wash tissue sample twice with PBS to remove fixative. Discard PBS.
2. Cut tissue fragment (up to 25 mg) into small pieces. Place the sample in 2 ml Eppendorf tube and add 200 µl of PBS.
3. Add 2 µl of **RNase A**, 20 µl of **Proteinase K** and 80 µl **Lyse T**. Mix by inverting or vortexing the tube.
4. Incubate at 56°C until the tissue is completely lysed (at least 1-3 h). Mix by inverting or vortexing every 15-30 min.
    - *Samples can be lysed overnight, if needed.*
5. Follow the point 1. Part II. of the DNA isolation protocol.

D. Liquid tissues/body fluids

(blood, saliva, plasma, serum, brain-spinal cord liquid among others)

1. To 100 µl of sample add 2 µl **RNase A** and 100 µl PBS.
    - *For sample volumes less than 100 µl, add PBS to adjust the volume to 100 µl.*
2. Add 20 µl **Proteinase K** and 80 µl **Lyse T**. Mix by vortexing or pipetting.
3. Incubate for 15 min at room temperature.
4. Follow the point 1. Part II. of the DNA isolation protocol.

E. Saliva/swab

1. To 200 µl of sample add 2 µl **RNase A**, 80 µl **Lyse T** and 10 µl **Proteinase K**.
   - *In case of dry swabs: add 300 µl PBS into the sample, mix and incubate for at least 5 min. Transfer 200 µl of sample to the new tube.*
   - *In case of swabs in storage buffer (with guanidine) transfer 200 µl of sample to the new tube.*
2. Mix thoroughly by vortexing the tube.
3. Incubate for 15 min at room temperature.
4. Follow the point 1. Part II. of the DNA isolation protocol.

F. Semen

1. Add 1.5 ml 10 mM Tris pH 8.0 to 100 µl of semen, mix for 10 min by vortexing.
2. Spin down for 5 min with 10 000 x g.
3. Remove the supernatant and resuspend the pelet in 200 µl PBS.
4. Add 2 µl **RNase A**, 20 µl **Proteinase K**, 80 µl **Lyse T** and 10 µl 1 M DTT.
5. Mix and incubate at 56°C until the tissue is completely lysed (at least 1 h).
   - *Samples can be lysed overnight, if needed.*
6. Follow the point 1. Part II. of the DNA isolation protocol.

G. Cultured cells

1. Centrifuge the cell culture (up to 10^7 cells) in the 1.5–2 ml Eppendorf tube for 3 min at 1 000 x g.
2. Carefully discard the supernatant. Add to the pellet 200 µl PBS, 80 µl **Lyse T**, 10 µl **Proteinase K** and 2 µl **RNase A**. Suspend the cells thoroughly by vortexing for 20 sec.
3. Incubate for 15 min at room temperature.
4. Follow the point 1. Part II. of the DNA isolation protocol.

H. Hair

1. Cut off the hair roots from the hair sample (up to 100 roots or 25 mg). Place them in the 2 ml Eppendorf tube. Add 200 µl of PBS, 80 µl **Lyse T**, 20 µl of 1M DTT and 20 µl of **Proteinase K**. Mix by vortexing.
   - *If the hair sample doesn't contain the roots cut the hair stems into short pieces not longer than 0.5 cm.*
   - *The hair stem is the dead part of hair that contain small quantities of degraded DNA. The recommended amplicon length for PCR analysis of DNA from the hair stems is <200 bp.*
2. Incubate at 56°C until the hair sample is completely lysed (6-8 h or overnight).
3. Mix by vortexing every 1-2 h or use a shaking water bath.
4. Spin down for 3 min with the maximal speed.
   - *This step removes undigested tissue fragments.*
5. Follow the point 1. Part II. of the DNA isolation protocol.

I. Urine

1. Add 2 ml of urine to the 2 ml Eppendorf tube.
2. Centrifuge urine in microcentrifuge for 2 min at 6 000 x g.
3. Carefully discard the supernatant without disturbing the pellet. Add to the pellet 200 µl PBS, 80 µl **Lyse T** and 10 µl **Proteinase K**.
4. Vortex for 15 sec.
5. Incubate for 60 min at 56°C, mix by inverting the tube every 15 min.
6. Follow the point 1. Part II. of the DNA isolation protocol.

Part II DNA isolation

1. Add 500 µl of buffer **Sol T** and mix thoroughly by vortexing or several-fold inverting.

   - *If working on 96-well plate format transfer the samples to the wells of the plate.*

2. Resuspend **Magnetic Beads** before removing them from the storage tube by vortexing. Add 10 µl of resuspended **Magnetic Beads** to the sample and mix by vortexing or pipetting for 1 min.

3. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

4. Remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 400 µl of **Wash T1** and mix by pipetting or vortexing for 10 s.

5. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

6. Remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash T2** and mix by pipetting or vortexing for 10 s.

7. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

8. Remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash T3** and mix by pipetting or vortexing for 10 s.

9. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

10. Remove and discard the supernatant by pipetting. Leave the open tubes/plate in magnetic stand and air dry the beads for 15-20 min.

    - *Wash T3 contains alcohol, make sure all the solution evaporates before proceding to the next step.*

11. Add 50-200 µl **Elution** to the tube/well and mix by pipetting or vortexing for 5 min.

12. Separate the **Magnetic Beads** against the side of the wells. After all the beads have been attached to the magnets transfer the supernatant containing the purified DNA to a suitable tube/plate. DNA is ready for analysis/manipulations. Isolated DNA can be stored either at 2-8° or at -20°.

Appendix 1 DNA isolation from bacterial cells

NOTE 1 • It is recommended to use this protocol for genomic DNA isolation from bacteria cultures, suspensions and tissues/body fluids containing bacteria.

NOTE 2 • Lysozyme containing BL buffer (E0309-02) is not included in this kit, it has to be ordered seperately. While ordering please indicate the buffer name and catalog number. Certain bacterial species are resistant to lysis, thus supplementary enzymes other than lysozyme may be necessary. For example, lysis of Staphylococcus is much more efficient with lysostaphin.

NOTE 3 • Tissue digestion with Lisozyme (BL buffer) and Proteinase K can be performed in the room temperature (56°C recommended) prolonging the incubation time.

1. Mix in 1.5 ml Eppendorf tube:
   a) 150 µl overnight bacterial culture and 80 µl **Lyse T** buffer.
   b) Pick bacterial colony directly from Petri dish and suspend in 150 µl buffer PBS and 80 µl **Lyse T**.
   c) Pellet bacteria from 0.1–1.5 ml overnight culture by centrifugation and discard the supernatant, ensuring that all liquid is completely removed. Resuspend the bacterial pellet in 150 µl PBS and 80 µl **Lyse T**.
      - *For high yield isolation it is critical to completely resuspend bacterial cells.*
      - *The highest quality DNA is obtained from bacterial culture, which are either in log phase or early stationary phase.*
2. Add 50 µl buffer **BL** and 2 µl **RNase A** to the suspension cell (point 1). Mix by several-fold inverting or vortex 3 sec.

   - *For efficient lysis of some bacterial species, enzymes other than lysozyme may be necessary. Use the appropriate enzyme (with buffer BL) for the particular species.*
3. Incubate the sample at 37°C for 15 min.
4. Add 20 µl **Proteinase K** to the resuspended cell pellet. Mix by several-fold inverting or vortex 3 sec.
5. Incubate the sample at 56°C for 30 min.
6. Follow the point 1. Part II. of the DNA isolation protocol.

Safety Information

Lyse T

Warning

H302+H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P261 Avoid breathing vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312 If swallowed: call a poison center/ doctor if you feel unwell.
P304+P340 If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333+P313 If skin irritation or rash occurs: get medical advice/attention.
P337+P313 If eye irritation persists: get medical advice/ attention.
EUH208 Contains ethylenediammonium dichloride. May produce an allergic reaction.

Proteinase K

Danger

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261 Avoid breathing vapours/spray.
P304+P340 If inhaled: remove person to fresh air and keep comfortable for breathing.
P342+P311 If experiencing respiratory symptoms: call a poison center or doctor/ physician.

Sol T

Danger

H226 Flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+P235 – Store in a well-ventilated place. Keep cool.

Wash T1

Danger

H226 Flammable liquid and vapour.
H302+H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312 If swallowed: call a poison center/ doctor if you feel unwell.
P302+P352 If on skin: wash with plenty of water.
P304+P340 If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Wash T2 / Wash T3

Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+P235 Store in a well-ventilated place. Keep cool.
P337+P313 If eye irritation persists: get medical advice/ attention.

- ***GeneMAGNET Human and Animal Tissue DNA Purification Kit is designed for rapid manual or automatic purification of total DNA (genomic, mitochondrial) from a variety of tissues and biological liquids. Purified DNA is free of contaminants, such as: RNA, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others.***

  Tissues and cells are desintegraded in the presence of lysis buffers combination. Further, Proteinase K digests cellular proteins, including DNA binding proteins, among them nucleases. Optimized buffer and ethanol are added to provide selective conditions for DNA binding to Magnetic Beads. Traces of contaminants remaining in the solution are efficiently removed in three wash steps. High-quality cellular DNA is then eluted in low salt buffer, e.g.: Tris-HCl, TE or water. Isolated DNA is ready for downstream applications without the need for ethanol precipitation.

- **GeneMAGNET line is based on the use of silica paramagnetic beads (Magnetic Beads) for selective binding of RNA and DNA. The use of specially designed binding and washing buffers enables the efficient purification of highly pure nucleic acids.**

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