EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Tissue & Bacterial DNA Purification Kit

Kit for isolation of total DNA from human and animal tissues and bacteria

Cat. no. E3551

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Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for isolation of total DNA (genomic, mitochondrial) from a variety of tissues, biological liquids and from any a Gram +, Gram - bacteria. 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.To obtain maximum yield we also recommend specialized kits: for isolation of DNA from blood (QuickBlood DNA Purification Kit), cell culture (Cell Culture DNA Purification Kit) or biological traces (Swab Extract DNA Purification Kit and Bio-Trace DNA Purification Kit).

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg. The maximum volume of the column reservoir is 650 µl. One minicolumn enables purification of DNA from up to 25 mg solid tissues or 200 µl liquid tissues. Due to differences in growth characteristics of bacteria species, it is recommended to perform a preliminary experiment for determining the optimal starting amount. In general, the weight of the cell pellet should not exceed 50 mg per single minicolumn and the volume of the culture volume should not exceed 1.0 ml per single minicolumn.

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

NOTE 4 · 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. Tissue lysates are very sticky. This can lead to slow lysate filtration through the resin. Therefore it is advisible to check, if lysate and washes passed completely through the resin.

Content	50 preps E3551-01	150 preps E3551-02	Storage/Stability
Buffer T	1.8 ml	5.4 ml	15-25°C
Lyse T	21 ml	63 ml	15-25°C
Lyse BG	18 ml	54 ml	15-25°C
BL *	3 ml	9 ml	–20°C
RNase A (10 mg/ml)	0.12 ml	0.36 ml	2-8°C
Proteinase K (20 mg/ml)	1.2 ml	3.6 ml	–20°C
Sol T	21 ml	63 ml	15-25°C
Wash TX1	30 ml	90 ml	15-25°C
Wash TX2	30 ml	90 ml	15-25°C
Elution	18 ml	54 ml	15-25°C
DNA Binding Columns	50	3 x 50	15-25°C
Protocol	1	1	

* Contains lysozyme (20 mg/ml).

Equipment and reagents to be supplied by the experimenter

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes, a heating block capable of incubation at 37–70°C.
- [1 M] Dithiothreitol (DTT), ethanol [96–100% v/v], xylene and PBS. To prepare sterile PBS, dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml H_2O. Adjust pH to 7.4 with HCl. Add H_2O to 1 liter.

Protocol

I. DNA binding spin-column activation

1. Apply 30 µl of activation **Buffer T** onto the **DNA binding spin-column** (do not spin) and keep it at room temperature till transfering lysate to the spin-column (for best results at least 10 min).

   - *Addition of Buffer T onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.*
   - *The membrane activation should be done before starting isolation procedure.*

II. Sample preparation

A. Solid tissues

1. Tissue homogenization.

   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 350 µl of buffer **Lyse T** 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 100 µl PBS and homogenize the sample using a mechanical homogenizer. Add 250 µl of buffer **Lyse T**.

   c) Cut tissue fragment (up to 25 mg) into small pieces. Place the sample in 2 ml Eppendorf tube and add 350 µl of buffer **Lyse T**.

2. Add 2 µl of **RNase A** and 20 µl of **Proteinase K**. 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. Follow the point 1. Part III. 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 11 000 x g. Remove supernatant by pipetting.
4. Add 1 ml xylene to the pellet, mix by vortexing.
5. Centrifuge for 3 min at 11 000 x g. 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 11 000 x g. 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 350 µl of buffer **Lyse T**.
11. Follow the point 2. of A. Solid tissues 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 350 µl of buffer **Lyse T**.
3. Follow the point 2. of A. Solid tissues protocol.

D. Liquid tissues/body fluids

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

1. Add 2 µl **RNase A** to 200 µl liquid sample. Mix thoroughly by vortexing the tube.
    - *For sample volumes less than 200 µl, add PBS to adjust the volume to 200 µl.*
2. Incubate for 5 min at room temperature.
3. Add 10 µl **Proteinase K**.
4. Follow the point 1. Part III. of the DNA isolation protocol.

E. Cultured cells

1. Centrifuge the cell culture (up to 5 x 10^6 cells) in the 1.5–2 ml Eppendorf tube for 2 min at 1 000 x g.
2. Carefully discard the supernatant. Add to the pellet 200 µl of **Lyse T** buffer and 2 µl of **RNase A**. Suspend the cells thoroughly by vortexing for 20 sec.
3. Incubate for 5 min at room temperature.
4. Add 10 µl **Proteinase K**.
5. Follow the point 1. Part III. of the DNA isolation protocol.

F. Rodent tails

1. Cut up to 1.2 cm-piece of mouse tail or up to 0.6 cm-piece of rat tail into the 2 ml Eppendorf tube. Add 350 µl of buffer **Lyse T**.
2. Add 2 µl of **RNase A** and 20 µl of **Proteinase K**. Mix by vortexing.
3. Incubate at 56°C until the tissue is completely lysed. Mix by vortexing every 1 h or use a shaking water bath.
   - *Samples can be lysed overnight.*
4. Vortex for 15 sec. Centrifuge for 3 min at 11 000 x g. Transfer the supernatant into a new tube.
   - *This step removes residual bones and hair.*
5. Add 350 µl of buffer **Sol T**. Add 350 µl of ethanol (96–100%). Mix thoroughly by vortexing.
6. Follow the point 6. Part III. of the DNA isolation protocol.

G. 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 350 µl of buffer **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. Follow the point 1. Part III. of the DNA isolation protocol.

H. Insects

1. Instect homogenization.

   a) Grind insects under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place the powder (up to 50 mg) in 2 ml Eppendorf tube. Centrifuge the sample to the bottom of the tube. Add 350 µl of buffer **Lyse T** 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 50 mg insects in 2 ml Eppendorf tube. Add 100 µl PBS and homogenize the sample using a mechanical homogenizer. Add 250 µl of buffer **Lyse T**.

2. Follow the point 2. of A. Solid tissues 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 350 µl **Lyse T** buffer 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 III. of the DNA isolation protocol.

J. Bacteria

1. Mix in 1.5 ml Eppendorf tube:

   a) 100 µl overnight bacterial culture and 200 µl **Lyse BG** buffer.

   b) Pick bacterial colony directly from Petri dish and suspend in 300 µl buffer **Lyse BG**.

   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 300 µl buffer **Lyse BG**.

      - *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. Add 350 µl buffer **Sol T**. Mix by several-fold inverting or vortex 3 sec.
7. Incubate the sample at 56°C for 5 min.
8. Vortex the sample for 15 sec.
9. Follow the point 5. part III of the DNA isolation protocol.

III. DNA isolation

1. Add 200 µl of buffer **Sol T** (D. Liquid tissues, E. Cultured cells) or 350 µl of buffer **Sol T** (A. Solid tissues, B. Paraffin-embedded tissues, C. Formalin-fixed tissues, G. Hair, H. Insects, I. Urine) and mix thoroughly by vortexing or several-fold inverting.
2. Incubate for 10 min at 70°C.
3. Add 200 µl of ethanol (96–100%) (D. Liquid tissues, E. Cultured cells) or 350 µl of ethanol (96–100%) (A. Solid tissues, B. Paraffin-embedded tissues, C. Formalin-fixed tissues, G. Hair, H. Insects, I. Urine).
4. Mix thoroughly by several times inverting the tube.
5. Centrifuge for 1 min at 12 000 x g.
6. Transfer the whole lysate (D. Liquid tissues, E. Cultured cells, J. Bacteria) or 600 µl of supernatant (A. Solid tissues, B. Paraffin-embedded tissues, C. Formalin-fixed tissues, F. Rodent tails, G. Hair, H. Insects, I. Urine) to the **DNA binding spin-column**, placed in the collection tube.
7. Centrifuge **DNA binding spin-column** for 1 min at 11 000 x g.

   - *Continue centrifugation at maximum speed, if not all of the lysate passed through the column.*

8. Remove the spin-column, discard flow-through and place back spin-column in the collection tube. In cases D. Liquid tissues and E. Cultured cells and J. Bacteria proceed with step 11.

9. Transfer the remaining supernatant (A. Solid tissues, B. Paraffin-embedded tissues, C. Formalin-fixed tissues, F. Rodent tails, G. Hair, H. Insects, I. Urine) to the **DNA binding spin-column**, placed in the collection tube. Repeat centrifugation for 2 min at 11 000 x g to pass completely the lysate through the resin.

   - *Continue centrifugation, if not all of the lysate passed through the column.*

10. Take out **DNA binding spin-column**, discard flow-through and place back spin-column in the collection tube.

11. Add 500 µl of **Wash TX1** buffer and centrifuge for 1 min at 11 000 x g.

12. Take out **DNA binding spin-column**, discard flow-through and place back spin-column in the collection tube.

13. Add 500 µl of **Wash TX2** buffer and centrifuge for 1 min at 11 000 x g.

14. Spin down at 11 000 x g for 1 min to remove traces of **Wash TX2** buffer.

15. Place **DNA binding spin-column** in a new collection tube (1.5–2 ml) and add 50–150 µl of **Elution** buffer to elute bound DNA.

    - *Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transferring traces of DNA between the spin-columns do not touch the spin-column walls with the micro-pipette.*

    - *In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.*

16. Incubate **DNA binding spin-column**/collection tube assembly for 2 min at room temperature.

17. Centrifuge for 1 min at 11 000 x g.

18. Remove spin-column, cap the collection tube. DNA is ready for analysis/manipulation. It can be stored either at 2–8°C or at -20°C.

Appendix 1:

Detection of Mycobacterium tuberculosis in sputum or bronchoalveolar lavage

1. Add 1 volume of NALC-NaOH solution (2% NaOH, 1.45% sodium citrate, 0.5% N-acetyl-L-cysteine) to 200–500 µl of sputum or bronchoalveolar lavage.

   - *To prepare NALC-NaOH solution dissolve: 2 g NaOH, 1.45 g sodium citrate, 0.5 g N-acetyl-L-cysteine. Add sterile distilled water to 100 ml.*

2. Mix by vortexing and incubate for 20 min at room temperature. Mix by vortexing or inverting every 5 min.
3. Adjust the volume to 25 ml with sterile distilled water.
4. Centrifuge for 30 min at 4000 x g. Discard the supernatant.
5. Resuspend the pellet in 0.5–1 ml of buffer **Lyse T**.
6. Transfer 200 µl of the sample to a new microcentrifuge tube.
7. Add 20 µl **Proteinase K**. Mix by vortexing or inverting the tube.
8. Incubate for 1 hr at 56°C. Mix by vortexing or inverting every 15 min.
9. Follow the point 1. Part III. DNA isolation according to protocol for: D. Liquid tissues, E. Cultured cells.

Safety Information

Buffer T

Danger

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331 If swallowed: Rinse mouth. Do not induce vomiting.
P303+P361+P353 If on skin (or hair): take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a poison center/doctor.
P405 Store locked up.

Lyse BG / Lyse T

Warning

H319 Causes serious eye irritation.
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.
P337+P313 If eye irritation persists: Get medical advice/ attention.

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

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.

Wash TX1

Warning

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 TX2

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.

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

			ISOLATION OF DNA																			
			E3600	E3585	E3540	E3580	E3510	E3545	E3560	E3555	E3525	E3520	E3595	E3535	E3500	E3565	E3515	E3570	E3575	E3530	E3550	E3551
			MICELLULA DNA[2]	GRAM PLUS & YEAST GENOMIC DNA	AGAROSE – OUT DNA	BACTERIAL & YEAST GENOMIC DNA	BIO – TRACE DNA	BASIC DNA	BONE DNA	CELL CULTURE DNA	FOOD EXTRACT DNA	PCR / DNA CLEAN-UP	PLANT & FUNGI DNA	AGROBACTERIUM PLASMID DNA	PLASMID MINIPREP DNA	QUICK BLOOD DNA	SHORT DNA CLEAN-UP	SOIL DNA	STOOL DNA	SWAB-EXTRACT DNA	TISSUE DNA	TISSUE & BACTERIAL DNA
			AVAILABLE NUMBER OF ISOLATION (PREPS)																			
			50 150	25 100	50 150	50 150	25 100	50 150	25 50	50 150	25 100	50 150	50 150	50 150	50 150	50 150	25 100	50 100	50 100	25 100	50 150	50 150
DNA	GENOMIC	BACTERIA		●		●																●
		YEAST		●		●																
		CELL CULTURE								●											●	●
		PLANT											●									
		FUNGI											●									
		PLANT RICH IN POLYSACCHARIDES [1]											●									
		BLOOD														●						
		SOIL																●				
		STOOL																	●			
		SWAB																		●		
		ANIMAL TISSUES																			●	●
		FFPE TISSUE SECTIONS																			●	●
		RODENT TAILS																			●	●
		HAIR																			●	●
		INSECTS																			●	●
		URINE																			●	●
		BONE							●													
		BIOLOGICAL TRACES					●															
		FOOD									●											
	PLASMID	BACTERIA						●						●	●							
		YEAST				●																
	ISOLATION FROM AGAROSE GELS				●			●														
	PURIFICATION OF PCR PRODUCTS / DNA AFTER ENZYMATIC REACTIONS		●					●				●					●					

All kits contain buffers WASH in ready to use form

1. Additionally required lyse CT buffer (E0324)
2. Kit for creation of emulsions and subsequent DNA purification.

GeneMATRIX is synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures.

Novel binding and washing buffers are developed to take full advantage of GeneMATRIX capacity, yielding biologically active, high-quality nucleic acids. Matrix is conveniently pre-packed in ready-to-use spin-format. Unique chemical composition of the matrixes along with optimized construction of spin-columns improve the quality of final DNA or RNA preparation. To speed up and simplify isolation procedure, the key buffers are colour coded, which allows monitoring of complete solution mixing and makes purification procedure more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Such DNA or RNA can be directly used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybrydization among others. Additional advantage is reproducibility of matrix performance, as component preparation is carried at Eurx Ltd.

GeneMATRIX Tissue & Bacterial DNA Purification Kit is designed for rapid purification of total DNA (genomic, mitochondrial) from a variety of tissues, biological liquids and from a wide variety of bacterial physiological groups. Purified DNA is free of contaminants, such as: RNA, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others.

Sample is proteolitycally lysed in the presence of special buffer, aiding tissue and cells desintegration (aided by lysozyme in case of bacteria). Further, Proteinase K digests cellular proteins, including stripping-off DNA of all bound proteins, among them nucleases. Optimized buffer and ethanol are added to provide selective conditions for DNA binding during brief centrifugation, while contaminants pass through the GeneMATRIX resin in the spin-column. Traces of contaminants remaining on the resin are efficiently removed in two 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

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