EURx Molecular Biology Products

GeneMATRIX Tissue & Bacterial DNA Purification Kit

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

Cat. no. E3551

Version 1.0

July, 2009

For laboratory use only.
Not for drug, household or other uses.

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Note 1: The kit gives good results in isolation of DNA from various tissues and bacteria. 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: 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: One minicolumn enables purification of DNA from up to 25 mg solid tissues or 200 µl liquid tissues.

Note 4: 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 and Proteinase K and BL buffer at -20°C.

Note 5: 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.

Note 6: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

Note 7: The kit does not contain 1M DTT, 96 % ethanol, 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-columns activation

1. Apply 40 µl of activation **Buffer T** onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

Note 1: Addition of Buffer T onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.

Note 2: The membrane activation should be done before starting isolation procedure.

II. Sample preparation

A. Solid tissues

1.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.

Note 1: To obtain high yield of DNA a tissue fragment should be thoroughly grinded to a fine powder.

1.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**.

1.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.

Note 1: 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.

3. Incubate at room temperature for 15 min.

4. Centrifuge for 3 min at 12000 rpm.

5. Remove supernatant by pipetting.

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

7. Centrifuge for 3 min at 12000 rpm.

8. Remove supernatant by pipetting.

9. Add 1 ml **96 % ethanol** to the pellet. Mix by vortexing or inverting the tube.

10. Centrifuge for 3 min at 12000 rpm.

11. Remove supernatant by pipetting.

12. Repeat steps 9-11 once.

13. Incubate the open tube at 37°C until the ethanol has evaporated (app. 15 min).

14. Resuspend the tissue pellet in 350 µl of buffer **Lyse T**.

15. 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.

   Note 1: For sample volumes less than 200 µl, add PBS to adjust the volume to 200 µl.

2. Mix thoroughly by vortexing the tube.

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.

E. Cultured cells

1. Centrifuge cultured cells (up to 10^7 cells) in the 2 ml Eppendorf tube for 2 min at 5000 rpm.

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.

   Note 1: Samples can be lysed overnight.

4. Vortex for 15 seconds.

5. Centrifuge for 5 min at 14000 rpm.

   Note 1: This step removes residual bones and hair.

6. Transfer the supernatant into a new tube.

7. Add 350 µl of buffer **Sol T**. Add 350 µl of **96 % ethanol**. Mix thoroughly by vortexing.

8. 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**.

Note 1: If the hair sample doesn't contain the roots cut the hair stems into short pieces not longer than 0.5 cm.
Note 2: 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. Mix by vortexing.

3. Incubate at 56° C until the hair sample is completely lysed (6-8 h or overnight).

4. Mix by vortexing every 1-2 h or use a shaking water bath.

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

H. Insects

1.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.

Note 1: To obtain high yield of DNA a tissue fragment should be thoroughly grinded to a fine powder.

1.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 a microcentrifuge for 2 min at 8000 rpm.

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.

J. Bacteria

1. Mix in 1.5 ml Eppendorf tube:

   A. 100 µl overnight bacterial culture and 200 µl **Lyse BG** buffer.
   Or:
   B. Pick bacterial colony directly from Petri dish and suspend in 300 µl buffer **Lyse BG.**
   Or:
   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**.

   Note 1: For high yield isolation it is critical to completely resuspend bacterial cells.
   Note 2: 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 (p. **1.**) Mix by several-fold inverting or vortex 3 sec.

   Note 1: 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 **96 % ethanol** (D. Liquid tissues, E. Cultured cells) or 350 µl of **96 % ethanol** (A. Solid tissues, B. Paraffin-embedded tissues, C. Formalin-fixed tissues, G. Hair, H. Insects, I. Urine).

4. Mix thoroughly by vortexing or several times inverting the tube.

5. Centrifuge for 1 min at 12000 rpm.

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 spin-column, placed in the collection tube.

7. Centrifuge for 1 min at 12000 rpm.

Note 1: Continue centrifugation at 14000 rpm, if not all of the lysate passed through the column.

8. Take out the spin-column, discard flow-through and place back the 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 remainings of supernatant (A. Solid tissues, B. Paraffin-embedded tissues, C. Formalin-fixed tissues, F. Rodent tails, G. Hair, H. Insects, I. Urine) to the spin-column, placed in the collection tube. Repeat centrifugation for 2 min at.12000 rpm to filtrate the rest of the lysate through the resin.

Note 1: Continue centrifugation at 14000 rpm, if not all of the lysate passed through the column.

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

11. Add 500 µl of buffer **Wash TX1** to spin-column and centrifuge for 1 min at 12000 rpm.

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

13. Add 500 µl of buffer **Wash TX2** to spin-column and centrifuge for 2 min at 12000 rpm.

14. Place spin-column in a new collection tube (1.5-2 ml) and add 50-200 µl of Elution buffer (10 mM Tris-HCl, pH 8.5) heated to 70°C to elute bound DNA.

Note 1: Addition of eluting buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.
Note 2: The following eluting solutions can be used:
1. 5-10 mM Tris-HCl buffer, pH 8.0-9.0
2. 0.5-1 x TE buffer, pH 8.0-9.0 (not recommended for DNA sequencing).
3. Other special application buffers can be used, provided that their pH and salt concentration is similar to that of 5-10 mM Tris-HCl, pH 8.0-9.0.

15. Incubate spin-column/collection tube assembly for 3 min at room temperature.

16. Centrifuge for 1 min at 12000 rpm.

Optional:

17. Repeat elution once again as described in steps 14-16.

Note 1: This step improves DNA recovery from the column. A new collection tube can be used to prevent dilution of the first eluate or collection tube from step 14 can be reused to combine the eluates.
Note 2: More than 200 µl should not be used to elute into a single 1.5 ml microcentrifuge tube, as the spin-column will come into contact with the eluate, causing DNA contamination.

18. Discard spin-column, cap the collection tube. Genomic 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.

   Note 1: 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.

Nucleic Acids Purification Systems

	Cat.No.	Package
GeneMATRIX AGAROSE - OUT DNA Purification Kit	E3540-01	50 preps
Kit for purification of DNA from agarose gels.	E3540-02	150 preps
GeneMATRIX BACTERIAL & YEAST GENOMIC DNA Purification Kit	E3580-01	50 preps
Kit for purification of DNA from Gram-positive and Gram-negative bacteria, yeast.	E3580-02	150 preps
GeneMATRIX BASIC DNA Purification Kit	E3545-01	50 preps
Universal kit for purification of PCR products / DNA after enzymatic reactions, isolation of DNA from agarose gels and isolation of plasmid DNA from bacteria.		
GeneMATRIX BIO-TRACE DNA Purification Kit	E3510-01	25 preps
Kit for purification of DNA from various samples for clinical and forensic analysis.	E3510-02	100 preps
GeneMATRIX BONE DNA Purification Kit	E3560-01	25 preps
Kit for isolation of DNA from animal or human bones.	E3560-02	100 preps
GeneMATRIX CELL CULTURE DNA Purification Kit	E3555-01	50 preps
Kit for purification of DNA from human and animal cell cultures.	E3555-02	150 preps
GeneMATRIX FOOD DNA Purification Kit	E3525-01	25 preps
Kit for purification of DNA from food.	E3525-02	100 preps
GeneMATRIX HUMAN BLOOD RNA Purification Kit	E3596-01	25 preps
Kit for isolation of total RNA from fresh human blood.		
GeneMATRIX PCR / DNA CLEAN - UP DNA Purification Kit	E3520-01	50 preps
Kit for purification of PCR products / DNA after enzymatic reactions.	E3520-02	150 preps
GeneMATRIX PLANT & FUNGI DNA Purification Kit	E3595-01	50 preps
Kit for purification of total DNA from plants, fungi and lichens.	E3595-02	150 preps
GeneMATRIX PLASMID MINIPREP DNA Purification Kit	E3500-01	50 preps
Kit for isolation of high-purity plasmid DNA (1.5-4 ml bacterial culture).	E3500-02	150 preps
GeneMATRIX QUICK BLOOD DNA Purification Kit	E3565-01	50 preps
Kit for quick purification of DNA from fresh or frozen blood.	E3565-02	150 preps
GeneMATRIX SHORT DNA Clean-Up Purification Kit	E3515-01	25 preps
Kit for purification of short single-stranded and double-stranded DNA fragments after enzymatic reactions	E3515-02	100 preps
GeneMATRIX SOIL DNA Purification Kit	E3570-01	50 preps
Kit for purification of DNA from soil.	E3570-02	100 preps
GeneMATRIX STOOL DNA Purification Kit	E3575-01	50 preps
Kit for purification of DNA from stool samples.	E3575-02	100 preps
GeneMATRIX SWAB EXTRACT DNA Purification Kit	E3530-01	25 preps
Kit for purification of DNA from swabs for clinical and forensic analysis.	E3530-02	100 preps
GeneMATRIX TISSUE DNA Purification Kit	E3550-01	50 preps
Kit for purification of DNA from human and animal tissues.	E3550-02	150 preps
GeneMATRIX TISSUE & BACTERIAL DNA Purification Kit	E3551-01	50 preps
Kit for purification of DNA from human and animal tissues, cell cultures and bacteria.	E3551-02	150 preps
GeneMATRIX UNIVERSAL DNA/RNA/Protein Purification Kit	E3597-01	25 preps
Kit for purification of total DNA/RNA/Protein from the same biological sample.	E3597-02	100 preps
GeneMATRIX UNIVERSAL RNA Purification Kit	E3598-01	25 preps
Kit for purification of total RNA from tissues, plants, bacteria, yeast and cell cultures.	E3598-02	100 preps
GeneMATRIX UNIVERSAL RNA/miRNA Purification Kit	E3599-01	25 preps
Kit for isolation of total RNA and miRNA from the tissues, plants and cell cultures.	E3599-02	100 preps
MICELLULA DNA Emulsion & Purification Kit	E3600-01	50 preps
For Emulsion PCR and other DNA targeted enzymatic reactions.	E3600-02	150 preps

SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL			AGAROSE – OUT	BACTERIAL & YEAST GENOMIC	BIO – TRACE	BASIC	BONE	CELL CULTURE	FOOD	HUMAN BLOOD RNA	PCR / DNA CLEAN-UP	PLANT & FUNGI	PLASMID MINIPREP	QUICK BLOOD	SHORT / DNA CLEAN-UP	SOIL	STOOL	SWAB EXTRACT	TISSUE	TISSUE & BACTERIAL	UNIVERSAL DNA/RNA /PROTEIN	UNIVERSAL RNA	UNIVERSAL RNA /miRNA	MICELLULA DNA
DNA	GENOMIC	BACTERIA		X																X				
		YEAST		X																				
		CELL CULTURE						X											X	X				
		PLANT AND FUNGI										X												
		BLOOD												X										
		SOIL														X								
		STOOL															X							
		SWAB																X						
		SOLID TISSUES																	X	X				
		LIQUID TISSUES																	X	X				
		RODENT TAILS																	X	X				
		HAIR																	X	X				
		INSECTS																	X	X				
		URINE																	X	X				
		BONE					X																	
		BIOLOGICAL TRACES			X																			
		FOOD							X															
	PLASMID	BACTERIA				X							X											
		YEAST		X																				
	ISOLATION FROM AGAROSE GELS		X			X																		
	PURIFICATION OF PCR PRODUCTS/DNA AFTER ENZYMATIC REACTIONS					X					X				X									X
DNA/RNA/PROTEIN FROM THE SAME BIOLOGICAL SAMPLE		ANIMAL TISSUE																			X			
		PLANT TISSUE																			X			
		BACTERIA																			X			
		YEAST																			X			
		CELL CULTURE																			X			
RNA	TOTAL RNA LONGER THAN 200 BASES	ANIMAL TISSUE																				X		
		PLANT TISSUE																				X		
		BACTERIA																				X		
		YEAST																				X		
		CELL CULTURE																				X		
		HUMAN BLOOD								X														
	miRNA AND TOTAL RNA	ANIMAL TISSUE																					X	
		PLANT TISSUE																					X	
		CELL CULTURE																					X	

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 DNA Purification Kit is designed for rapid 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.

Sample is proteolitycally lysed in the presence of special buffer, aiding tissue and cells desintegration. 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.