EURx Molecular Biology Products

GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit

Universal kit for isolation of total DNA from bacteria Gram $^{+}$, Gram^{-} and yeast.

Cat. no. E3580

Version 1.1

September, 2008

Note: Store binding spin-columns at 2÷8°C.

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

EURx Ltd. 80-297 Gdansk Poland ul. Przyrodnikow 3, NIP 957-07-05-191, KRS 0000202039,
www.eurx.com.pl orders: *email*: orders@eurx.com.pl *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Note 1: This kit is designed for isolation of DNA from any a Gram $^{+}$, Gram^{-} bacteria and from yeast.

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: For efficient lysis of yeast species zymolase or lyticase is necessary.

Note 4: Once the kit is unpacked, store components at room temperature, with the exception of BL buffer (with lysozyme) and Proteinase K, which should be kept at -20°C. RNase A and binding spin columns store at 2÷8°C.

Note 5: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

Protocol

I. 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. Follow the point 5. part III of the DNA isolation protocol.

II. Yeast

1. Pellet yeast cells from an appropriate volume of culture (weight of pellet should not exceed 100 mg) by centrifugation and discard the supernatant, ensuring that all liquid is completely removed. Resuspend the yeast pellet in 300 µl buffer **Lyse BG**.

   Note 1: For high yield isolation it is critical to completely resuspend yeast cells.

   Note 2: Due to the different growth characteristics of yeast species, performing a preliminary experiment to determine the optimal starting volume is recommended. Weight of pellet should not exceed 100 mg per one minicolumn.

   Note 3: Add 1 µl β-mercaptoethanol (β-ME) per 1 ml Lyse BG before use. Lyse BG is stable for 1 month after addition of β-ME.

2. Centrifuge for 1 min at 11000 x g, discard the supernatant and again resuspend the yeast pellet in 250 µl buffer **Lyse BG**. Mix as thoroughly as possible by pipetting.

3. Add appropriate enzyme (for example lyticase) and 2 µl **RNase A** to the resuspended pellet. Mix well and incubate at 30°C for 30 min.

   Note 1: 50 U lyticase/zymolase per 1 x 10^7 cells. Maximum volume of added enzyme can not exceed 50 µl.

4. Follow the point 5. part III of the DNA isolation protocol.

III DNA isolation

5. Add 15 µl **Proteinase K** to the resuspended cell pellet. Mix by several-fold inverting or vortex 3 sec.

6. Incubate the sample at 55°C for 30 min.

7. Add 350 µl buffer **Sol BG.** Mix by several-fold inverting or vortex 3 sec.

8. Incubate the sample at 55°C for 5 min.

9. Vortex the sample for 15 sec.

10. Centrifuge the lysate for 2 min at 11000 x g, and transfer the supernatant to a spin-column.

11. Centrifuge for 1 min at 11000 x g.

12. Remove the spin-column, pour off supernatant and place back into the receiver tube.

13. Add 600 µl of **Wash BGX** buffer and centrifuge at 11000 x g for 1 minute.

14. Remove the spin-column, pour off supernatant and place back into the receiver tube.

15. Add 300 µl of **Wash BGX** buffer and spin down at 11000 x g for 2 minutes.

16. Place spin-column into new receiver tube (1.5-2 ml) and add 100 µl of **Elution** buffer heated to 80°C to elute bound DNA.

Note 1: Addition of eluting buffer directly onto the center of the membrane improves DNA yield.

Note 2: The following eluting solutions can be used:
1. 5-10 mM Tris-HCl buffer, pH 7.5-8.5 (recommended when DNA is to be stored for prolonged periods at -20°C).
2. High quality distilled water (pH 7.5-8.5).
3. 0.5-1 x TE buffer (not recommended for DNA sequencing).
4. 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 7.5.

17. Incubate spin-column/receiver tube assembly for 2 minutes at room temperature.

18. Centrifuge for 2 min at 11000 x g.

19. Remove spin-column, cap the receiver tube. Genomic DNA is ready for analysis/manipulations. It can be stored either at 2÷8° C (preferred) or at -20°C (avoid multiple freezing and defrosting of DNA).

Appendix:

Yeast genomic and plasmid DNA Purification Protocol

Note 1: This appendix is for simultaneous isolation of genomic and plasmid DNA from yeast.

Note 2: For efficient lysis of yeast species zymolase or lyticase is necessary.

Note 3: Once the kit is unpacked, store components at room temperature, with the exception of BL buffer (with lysozyme) and Proteinase K, which should be kept at -20°C. RNase A and binding spin columns store at 2÷8°C.

Note 4: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

Protocol

1. Apply 40 µl of activation **Buffer BG** onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column (for best results at least 20 min).

   Note 1: Addition of Buffer BG 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.

2. Pellet yeast cells from an appropriate volume of culture (weight of pellet should not exceed 100 mg) by centrifugation and discard the supernatant, ensuring that all liquid is completely removed. Resuspend the yeast pellet in 300 µl buffer **Lyse BG**.

   Note 1: For high yield isolation it is critical to completely resuspend yeast cells.

   Note 2: Due to the different growth characteristics of yeast species, performing a preliminary experiment to determine the optimal starting volume is recommended. Weight of pellet should not exceed 100 mg per one minicolumn.

   Note 3: Add 1 µl β-mercaptoethanol (β-ME) per 1 ml Lyse BG before use. Lyse BG is stable for 1 month after addition of β-ME.

3. Centrifuge for 1 min at 11000 x g, discard the supernatant and again resuspend the yeast pellet in 225 µl buffer **Lyse BG**. Mix as thoroughly as possible by pipetting.

4. Add appropriate enzyme (for example lyticase) and 2 µl **RNase A** to the resuspended pellet. Mix well and incubate at 30°C for 30 min.

   Note 1: 50 U lyticase/zymolase per 1 x 10^7 cells. Maximum volume of added enzyme can not exceed 50 µl.

5. Add 15 µl **Proteinase K** to the resuspended cell pellet. Mix by several-fold inverting or vortex 3 sec.

6. Incubate the sample at 55°C for 30 min.

7. Add 225 µl buffer **Sol BG.** Mix by several-fold inverting or vortex 3 sec.

8. ncubate the sample at 55°C for 5 min.

9. Vortex the sample for 15 sec.

10. Centrifuge the lysate for 2 min at 11000 x g, and transfer the supernatant to a new 2 ml tube.

11. Add 250 µl ethanol (96-100%) to the sample, and mix thoroughly by vortexing.

Note 1: A precipitate may form after addition of ethanol.

12. Apply the sample, including any precipitate, to the spin-column placed in a 2 ml collection tube.

13. Centrifuge for 1 min at 11000 x g.

14. Remove the spin-column, pour off supernatant and place back into the receiver tube.

15. Add 600 µl of **Wash BGX** buffer and centrifuge at 11000 x g for 1 minute.

16. Remove the spin-column, pour off supernatant and place back into the receiver tube.

17. Add 300 µl of **Wash BGX** buffer and spin down at 11000 x g for 2 minutes.

18. Place spin-column into new receiver tube (1.5-2 ml) and add 60-100 µl of **Elution** buffer heated to 80°C to elute bound DNA.

Note 1: Addition of eluting buffer directly onto the center of the membrane improves DNA yield.

Note 2: The following eluting solutions can be used:
1. 5-10 mM Tris-HCl buffer, pH 7.5-8.5 (recommended when DNA is to be stored for prolonged periods at -20°C).
2. High quality distilled water (pH 7.5-8.5).
3. 0.5-1 x TE buffer (not recommended for DNA sequencing).
4. 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 7.5.

19. Incubate spin-column/receiver tube assembly for 2 minutes at room temperature.

20. Centrifuge for 2 min at 11000 x g.

21. Remove spin-column, cap the receiver tube. Genomic DNA is ready for analysis/manipulations. It can be stored either at 2÷8°C (preferred) or at -20°C (avoid multiple freezing and defrosting of DNA).

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 Bacterial & Yeast Genomic DNA Purification Kit is designed for rapid purification of genomic DNA from a wide variety of bacterial physiological groups and from a wide variety of yeast strains. 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 lysed in the presence of special cell wall desintegrating buffer aided by lysozyme or in case of yeast by lyticase. Further, Proteinase K digests cellular proteins, including stripping-off DNA of all bound proteins, among them nucleases. Optimized buffer is added to provide selective conditions for DNA binding during brief centrifugation, while contaminants pass through the **GeneMATRIX** membrane in the spin-column. Traces of contaminants remaining on the membrane 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.