EURx® MOLECULAR BIOLOGY PRODUCTS

Version 1.1
September 2019

GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit

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

Cat. no. E3580

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Table of Contents

Content	50 preps E3580-01	150 preps E3580-02	Storage/Stability
Buffer BG	1.8 ml	5.4 ml	15-25°C
Lyse BG	33 ml	100 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)	0.9 ml	2.7 ml	–20°C
Sol BG	21 ml	63 ml	15-25°C
Wash BGX	55 ml	165 ml	15-25°C
Elution	6 ml	18 ml	15-25°C
DNA Binding Columns	50	3 x 50	15-25°C
Protocol	1	1	

* Contains lysozyme (20 mg/ml).

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for the rapid isolation of pure genomic DNA from any a Gram +, Gram - bacteria and from yeast. 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. For efficient lysis of yeast species zymolase or lyticase is necessary.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg. Use either stationary phase or log phase bacterial cultures for obtaining highest DNA quality. Due to differences in growth characteristics of bacteria and yeast 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. Do not use more than 1 x 10^9 yeast cells per single preparation. In case of minicolumn clogging due to high lysate viscosity, reduce the initial amount of bacteria or yeast used for isolation.

NOTE 3 · Kit Compounds Storage. 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. Store RNase A at 2–8°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.

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 30–55°C. For simultaneous isolation of genomic and plasmid DNA from yeast - ethanol [96–100% v/v]. For Yeast protocol: β mercaptoethanol (14.3 M, β ME).

Protocol

Part I Cell preparation and lysis.

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**.

   - *For obtaining maximum DNA yield, it is critical to completely resuspend the bacterial cell pellets.*
   - *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 pipetting 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. Continue with Part II of the Protocol.

Yeast

1. Pellet yeast cells from an appropriate volume of culture by centrifugation (the weight of pellet should not exceed 50 mg).
   Keep the pellet and discard the supernatant, ensuring that all liquid is completely removed. Resuspend the yeast pellet in 300 µl buffer **Lyse BG**.

   - *For obtaining maximum DNA yield, it is critical to completely resuspend yeast cells.*
   - *Due to the different growth characteristics of yeast species, it is recommended to perform a preliminary experiment for determining the optimal starting volume. The weight of pellet should not exceed 50 mg per one minicolumn. Do not use more than 1 x 10^9 yeast cell per one single prep.*

- *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 11 000 x g, discard the supernatant and resuspend the yeast pellet again 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.

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

4. Continue with Part II of the Protocol.

Part II DNA isolation.

1. Add 15 µl **Proteinase K** to the resuspended cell pellet. Mix by several-fold inverting or vortex 3 sec.
2. Incubate the sample at 55°C for 30 min.

   - *During the incubation period, mix by occasionally inverting the tube several times.*

3. Add 350 µl buffer **Sol BG**. Mix by several-fold inverting or vortex 3 sec.
4. Incubate the sample at 55°C for 5 min.
5. Vortex the sample for 15 sec.
6. Centrifuge the lysate for 2 min at 11 000 x g, and carefully transfer up to 600 µl of a clear supernatant to a **DNA binding spin-column**.
7. Centrifuge for 1 min at 11 000 x g. Remove the spin-column, discard the flow-through and stick the spin column back onto the collection tube.
8. Transfer the remaining mixture to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
9. Add 450 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 1 min.
10. Remove the spin-column, discard the flow-through and stick the spin column back onto the collection tube.
11. Add 450 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 1 min.
12. Remove the spin-column, discard the flow-through and stick the spin column back onto the collection tube.

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

14. Place spin-column into new receiver tube (1.5–2 ml) and add 50–100 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.*

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

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

17. 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

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

1. Apply 30 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 15 min).

   - *Addition of Buffer BG 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.*

2. Pellet yeast cells from an appropriate volume of culture (weight of pellet should not exceed 50 mg) by centrifugation and discard the supernatant, ensuring that all liquid is completely removed. Resuspend the yeast pellet in 300 µl buffer **Lyse BG**.

   - *For high yield isolation it is critical to completely resuspend yeast cells.*

   - *Due to the different growth characteristics of yeast species, it is recommended to perform a preliminary experiment for determining the optimal starting volume. The weight of pellet should not exceed 50 mg per one minicolumn. Do not use more than 1 x 10^9 yeast cell per one single prep.*

   - *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 11 000 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.

   - *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. Incubate the sample at 55°C for 5 min.

9. Vortex the sample for 15 sec.

10. Centrifuge the lysate for 2 min at 11 000 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.

    - *A precipitate may form after addition of ethanol.*

12. Apply up to 600 µl of sample, including any precipitate, to **DNA binding spin-column** placed in a 2 ml collection tube.

13. Centrifuge for 1 min at 11 000 x g. Remove the spin-column, discard the flow-through and stick the spin column back onto the collection tube.

14. Transfer the remaining mixture to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

15. Add 450 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

16. Add 450 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

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

18. Place spin-column into new receiver tube (1.5–2 ml) and add 50–100 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.*

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

20. Centrifuge for 1 min at 11 000 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).

Safety Information

Buffer BG

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

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 BG

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 BGX

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 were developed to take full advantage of GeneMATRIX capacity, yielding biologically active, high-quality nucleic acids. The matrix is conveniently pre-packed in ready-to-use spin-format. Due to the unique chemical composition of the matrices, in combination with optimized spin-column design, nucleic acids are isolated in outstanding quality and high purity. To speed up and simplify the isolation procedure, the key buffers are colour coded, allowing for monitoring complete mixing of mission-critical solutions, thus aiding to render the purification procedure even more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Isolated 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. One additional advantage is the high level of matrix performance reproducibility, as all components are prepared inhouse 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.

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