

## 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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<b>Content</b>	<b>50 preps E3580-01</b>	<b>150 preps E3580-02</b>	<b>Storage/Stability</b>
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  $1 \times 10^9$  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

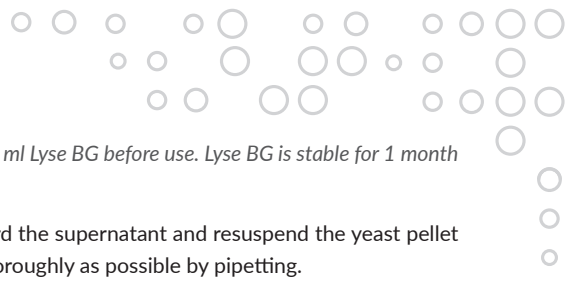
## Part I Cell preparation and lysis.

### Bacteria

1. Mix in 1.5 ml Eppendorf tube:
  - A. 100  $\mu$ l overnight bacterial culture and 200  $\mu$ l **Lyse BG** buffer.  
**Or:**
  - B. Pick bacterial colony directly from Petri dish and suspend in 300  $\mu$ l 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  $\mu$ l 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  $\mu$ l buffer **BL** and 2  $\mu$ l **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  $\mu$ l 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 micocolumn. Do not use more than  $1 \times 10^9$  yeast cell per one single prep.

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- Add 1  $\mu$ l  $\beta$ -mercaptoethanol ( $\beta$ -ME) per 1 ml Lyse BG before use. Lyse BG is stable for 1 month after addition of  $\beta$ -ME.
  - 2. Centrifuge for 1 min at 11 000 x g, discard the supernatant and resuspend the yeast pellet again in 250  $\mu$ l buffer **Lyse BG**. Mix as thoroughly as possible by pipetting.
  - 3. Add appropriate enzyme (for example lyticase) and 2  $\mu$ l **RNase A** to the resuspended pellet. Mix well and incubate at 30°C for 30 min.
    - 50 U lyticase/zymolase per 1 x 10<sup>7</sup> cells. Maximum volume of added enzyme can not exceed 50  $\mu$ l.
  - 4. Continue with Part II of the Protocol.

## Part II DNA isolation.

1. Add 15  $\mu$ l **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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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.

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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  $\mu$ l of activation **Buffer BG** onto the spin-column (do not spin) and keep it at room temperature till transferring 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  $\mu$ l 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  $1 \times 10^9$  yeast cell per one single prep.
  - Add 1  $\mu$ l  $\beta$ -mercaptoethanol ( $\beta$ -ME) per 1 ml Lyse BG before use. Lyse BG is stable for 1 month after addition of  $\beta$ -ME.
3. Centrifuge for 1 min at 11 000 x g, discard the supernatant and again resuspend the yeast pellet in 225  $\mu$ l buffer **Lyse BG**. Mix as thoroughly as possible by pipetting.
4. Add appropriate enzyme (for example lyticase) and 2  $\mu$ l **RNase A** to the resuspended pellet. Mix well and incubate at 30°C for 30 min.
  - 50 U lyticase/zymolase per  $1 \times 10^7$  cells. Maximum volume of added enzyme can not exceed 50  $\mu$ l.
5. Add 15  $\mu$ l **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  $\mu$ l 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  $\mu$ l ethanol (96–100%) to the sample, and mix thoroughly by vortexing.
  - A precipitate may form after addition of ethanol.
12. Apply up to 600  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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.



**SELECTION OF THE KITS  
DEPENDING ON THE TYPE  
OF ISOLATED MATERIAL**

		ISOLATION OF DNA																					
		E3600	E3885	E3940	E3880	E3910	E3945	E3960	E3955	E3925	E3920	E3995	E3935	E3900	E3965	E3915	E3970	E3975	E3930	E3950	E3951		
		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 <sup>1</sup>												●									
		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, hybridization 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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