EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Gram Plus & Yeast Genomic DNA Purification Kit

Universal kit for isolation of total DNA from gram positive bacteria, yeast and microorganisms present in small arachnids/insects. The kit contains glass beads for mechanical cell disruption.

Cat. no. E3585

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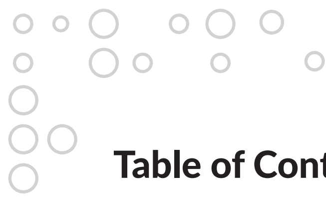

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Introductory Notes

NOTE 1 • Kit Specification. The kit is designed for the rapid isolation of pure genomic DNA from any species of gram positive bacteria and from yeast. The kit also allows the isolation of DNA from microorganisms present in small arachnids/insects (e.g. *Borrelia burgdorferi* from ticks). You should then proceed in accordance with Appendix 1 page 9.

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. Lyse BGplus buffer contains certain buffer components which are susceptible to separate into phases. Shake buffer Lyse BGplus well before use.

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.

The kit contains glass beads for efficient mechanical cell disintegration. Due to the broad biological diversity in composition and construction of cell walls, certain gram positive bacteria and yeast species are very resistant to chemical and enzymatic lysis. The kit uses a universal method of homogenization and cell lysis by combining mechanical trituration using glass beads of different diameters in an environment rich in detergents. To prevent excessive fragmentation of genomic DNA due to mechanical lysis treatment, special optimizations were applied to the lysis buffer, thus allowing to achieve DNA isolation with maximum yield and best possible integrity. Consequently, this method allows to achieve satisfactory efficiency of genomic DNA isolation without the use of any enzymes specific to the target group of bacteria or yeast. The GeneMatrix Gram Plus & Yeast Genomic DNA Purification Kit is a versatile system that allows to release and isolate genetic material from the whole broad spectrum of exceptionally lysis resistant organisms.

Content	25 preps E3585-01	100 preps E3585-02	Storage/Stability
Buffer BG	0.9 ml	3.6 ml	15-25°C
Lyse BG plus	20 ml	78 ml	15-25°C
BL *	1.5 ml	6 ml	–20°C
RNase A (10 mg/ml)	0.06 ml	0.24 ml	2-8°C
Proteinase K (20 mg/ml)	0.45 ml	1.8 ml	–20°C
Sol BG	12 ml	48 ml	15-25°C
Wash BGX	27 ml	108 ml	15-25°C
Elution	3 ml	12 ml	15-25°C
DNA Binding Columns	25	2 x 50	15-25°C
Bead Tube Dry	25	2 x 50	15-25°C
Filtration Columns	25	2 x 50	15-25°C
Protocol	1	1	

* Contains lysozyme (20 mg/ml).

Equipment and reagents to be supplied by the experimenter.

1. Equipment for sample disruption and homogenization: a flat-bed vortex pad or cell disrupter (FastPrep, Precellys, Disruptor Genie, etc.). Cell disruptors maximize DNA yields, but require careful optimization of shaking time (generally, a reduction compared to the time specified in the protocol).
2. Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5-2 ml tubes, a heating block capable of incubation at 37-70°C.
3. For DNA isolation from microorganisms present in insects (Appendix 1 page 9) - Tissue Grinding Tool cat. no. E0359 and ethyl alcohol [96-100% v/v].

Protocol

Part I Column preparation

1. Apply 30 µl of activation **Buffer BG** onto the **DNA binding spin-column** (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

- *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. The minimum activation time is 5-15 min.*

Part II Cell preparation

Bacteria

1. Pellet bacteria from a suitable culture volume (0.1-1.0 ml) by centrifugation. Discard the supernatant, ensuring that all liquid is completely removed. Resuspend the bacterial pellet in 300 µl buffer **Lyse BGplus**.

- *For obtaining maximum DNA yield, it is critical to completely resuspend the bacterial cell pellets. Some bacteria my form cell pellets which are rather difficult to suspend (e.g Streptomyces). In this case crush the pellet with great care to the smallest possible parts and create a homogenous solution.*

- *Lyse BGplus buffer contains components which may separate into phases. Shake buffer well before use.*

2. Add 50 µl buffer **BL** and 2 µl **RNase A** to the suspension cell (point 1) Mix by several-fold inverting or pipetting or vortex 3 sec.

3. Incubate the sample at 37°C for 15 min.

4. Continue with Part III of the Protocol.

Yeast

1. Centrifuge 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 200 µl buffer **Lyse BGplus**.

   - *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.*

   - *Lyse BGplus buffer contains components which may separate into phases. Shake buffer well before use.*

2. Centrifuge for 1 min at 11 000 x g, discard the supernatant and resuspend the yeast pellet again in 350 µl buffer **Lyse BGplus**. Mix as thoroughly as possible by pipetting.
3. Incubate the sample at 55°C for 15 min.
4. Continue with Part III of the Protocol.

Part III Lysis, homogenization and DNA isolation

1. Transfer the cell suspension to a **BeadTubeDry** with glass beads.

   - *Transfer may be achieved either by pouring or by pipetting.*

2. Secure **BeadTubeDry** horizontally to a vortex by using a vortex adapter or a tube holder. Vortex at maximum speed for 10 min.

   - *Alternatively, a cell disruptor (FastPrep, Precellys, Disruptor Genie, etc.) may be used. Maximum DNA yields are achieved by using a cell disruptor rather than by vortexing. But, for preventing excessive DNA fragmentation, it is required to optimize the shaking time (generally, a time reduction, as compared to the time specified above for vortexing, depending on the specific type of cell disruptor in use).*

   - *In case of strong foaming of the sample centrifuge BeadTubeDry for 30 sec at 5 000 x g.*

3. Add 15 µl **Proteinase K** to the resuspended cell pellet. Mix by several-fold inverting the tube.

   - *Add Proteinase K to BeadTubeDry containing glass beads and lysed cell suspension.*

4. Incubate **BeadTubeDry** at 55°C for 30 min.

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

5. Secure **BeadTubeDry** horizontally using a vortex adapter tube holder for the vortex. Vortex at maximum speed for 5 min.

   - *In case of yeast sample this step is not necessary. Continue with point 6.*

   - *In case of strong foaming of the sample centrifuge BeadTubeDry for 30 sec at 5 000 x g. After centrifugation mix by several-fold inverting the tube.*

6. Transfer the contents of the **BeadTubeDry** with glass beads to the **filtration spin-column** placed in a collection tube to recover the entire lysate.

   - *Incline the BeadTubeDry and pour contents to the filtration spin-column. During centrifugation, the lysate will be filtered and further homogenized.*

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

8. Remove the **filtration spin-column**. Add 380 µl **Sol BG** to the flow-through. Mix thoroughly by pipetting to resuspend any precipitate that may have formed during the spin down cycle. Transfer mixture to a new 1.5-2 ml Eppendorf-type tube.

9. Incubate at 55°C for 5 min.

10. Centrifuge at maximum speed for 2 min.

11. Carefully transfer the clear supernatant to a **DNA binding spin-column**.

    Pay attention not to stir up any pellet that may have formed during the spin down cycle.

12. 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.

13. Add 600 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 1 min.

14. Remove the spin-column, discard the flow-through and stick the spin column back onto the collection tube.

15. Add 300 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 2 min.

    Be careful not to contaminate the sample while removing the spin-column from the receiver tube. Check, whether the membrane of the spin column is completely dry. If not, pour off any remaining supernatant and stick back spin-column onto the receiver tube. Spin down for one additional min.

16. Place spin-column into new receiver tube (1.5-2 ml) and add 50-80 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.

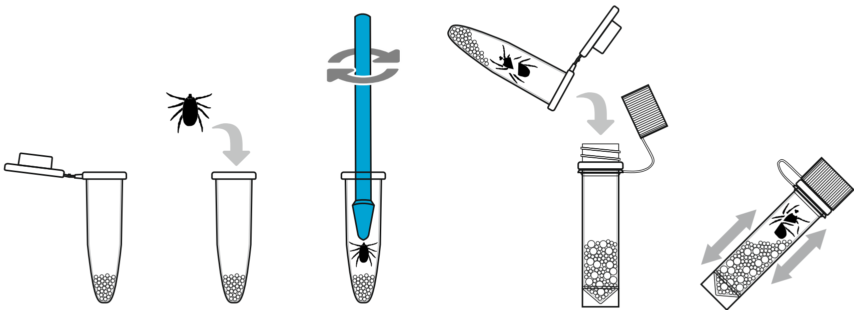
17. Incubate **DNA binding spin-column**/collection tube assembly for 2 min at room temperature.

18. Centrifuge for 2 min at 11 000 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 1: DNA isolation from microorganisms present in small arachnids/insects

The appendix is optimized for the isolation of DNA from microorganisms (bacteria, viruses, fungi) that live in small (up to 5 mm long) arachnids or insects. The first stage of isolation is the fragmentation of the arachnid/insect. In order to achieve this, use the Tissue Grinding Tool. It is a convenient tool for disrupting small portions of plant and animal tissues, bacterial, yeast or cell cultures pellets in quantities corresponding to one extraction experiment. The set consists of a 1.5 ml Eppendorf tube containing a small amount of grinding beads and a pestle with specially adapted shape. Arachnid/insect should be homogenized in a small amount of lysis buffer. The Tissue Grinding Tool is available separately, catalog number E0359. After preliminary fragmentation and release of microorganisms present in the host, proper homogenization of the mixture should be carried out. For this purpose, after digestion with Proteinase K, the contents of the tube should be transferred to BeadTubeDry (included in the kit) and shaken in a vortex or dedicated device (point 5).



1. Add 200 µl of **Lyse BGplus** buffer to the tube with selected grinding beads. Then add an arachnid/insect and crush by rotating the pestle with your fingers. The grinding time depends on the type of arachnid/insect and varies from 15 sec to a maximum of 1 min.

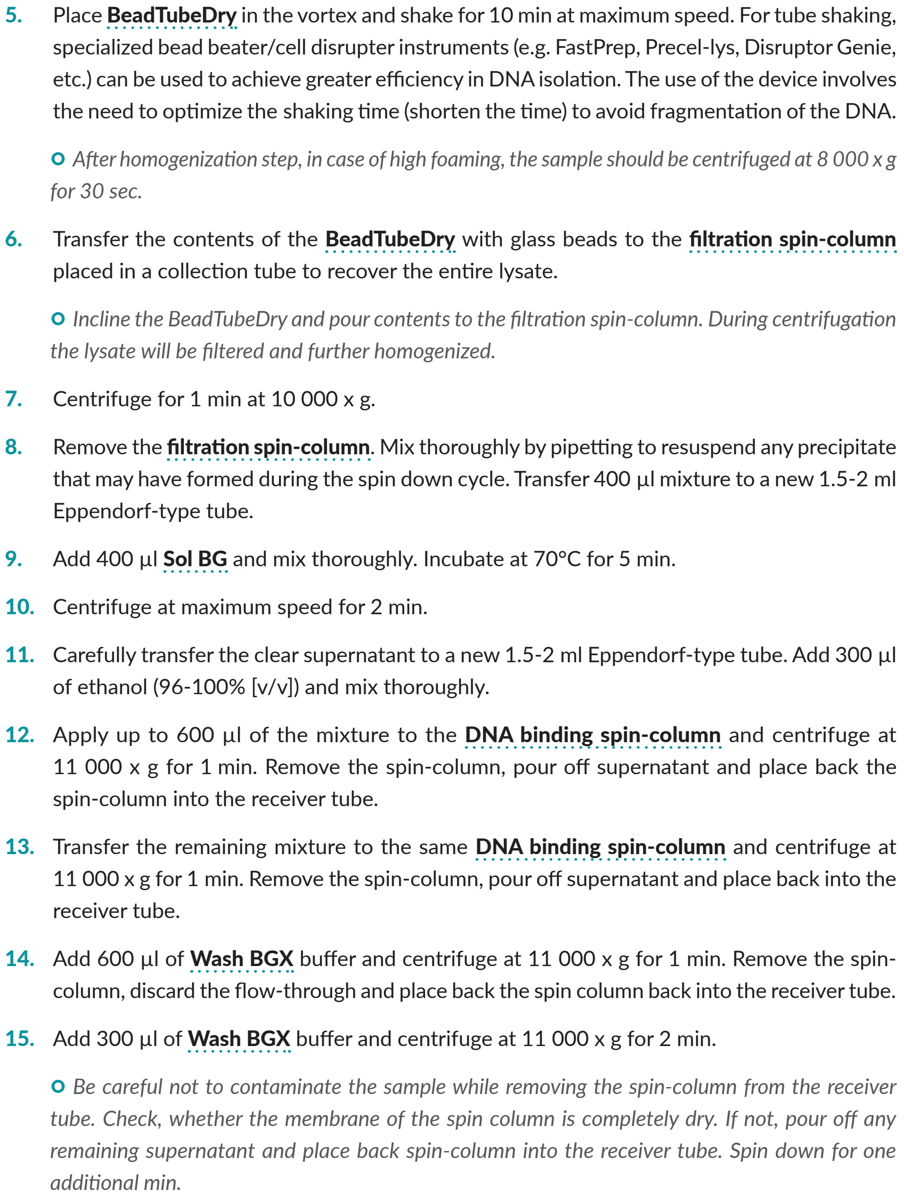
   - *For very small arachnid/insect (e.g. Ixodes ricinus) it is recommended to grind in a smaller volume of Lyse BGplus, e.g. 50 µl. In this case, before the addition of Proteinase K, the Lyse BGplus volume should be adjusted to 200 µl.*

   - *Activate the DNA binding spin-column as in Part I of the Protocol (see page 5).*

2. Remove the stick, add 15 µl **Proteinase K** and 2 µl **RNase A**. Vortex for a few seconds or mix thoroughly by inverting the tube.

3. Incubate at 60°C for 30 min.

4. Add 400 µl **Lyse BGplus** buffer and transfer the mixture to a **BeadTubeDry** with glass beads.



5. Place **BeadTubeDry** in the vortex and shake for 10 min at maximum speed. For tube shaking, specialized bead beater/cell disrupter instruments (e.g. FastPrep, Precel-lys, Disruptor Genie, etc.) can be used to achieve greater efficiency in DNA isolation. The use of the device involves the need to optimize the shaking time (shorten the time) to avoid fragmentation of the DNA.

   After homogenization step, in case of high foaming, the sample should be centrifuged at 8 000 x g for 30 sec.

6. Transfer the contents of the **BeadTubeDry** with glass beads to the **filtration spin-column** placed in a collection tube to recover the entire lysate.

   Incline the BeadTubeDry and pour contents to the filtration spin-column. During centrifugation the lysate will be filtered and further homogenized.

7. Centrifuge for 1 min at 10 000 x g.

8. Remove the **filtration spin-column**. Mix thoroughly by pipetting to resuspend any precipitate that may have formed during the spin down cycle. Transfer 400 µl mixture to a new 1.5-2 ml Eppendorf-type tube.

9. Add 400 µl **Sol BG** and mix thoroughly. Incubate at 70°C for 5 min.

10. Centrifuge at maximum speed for 2 min.

11. Carefully transfer the clear supernatant to a new 1.5-2 ml Eppendorf-type tube. Add 300 µl of ethanol (96-100% [v/v]) and mix thoroughly.

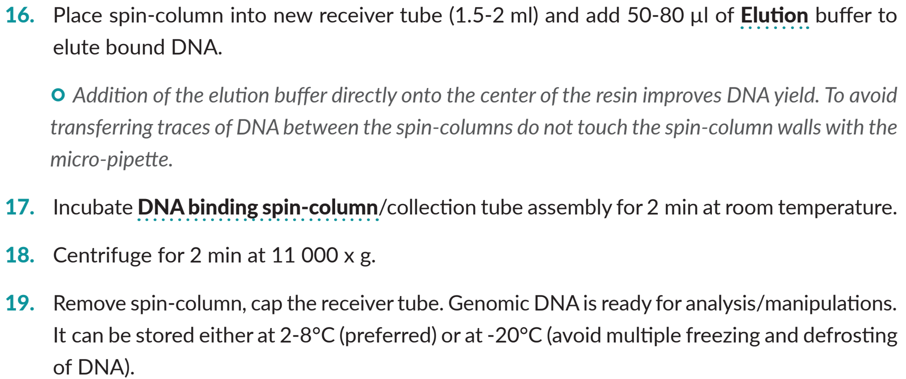
12. Apply up to 600 µl of the mixture to the **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back the spin-column into the receiver tube.

13. 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.

14. Add 600 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 1 min. Remove the spin-column, discard the flow-through and place back the spin column back into the receiver tube.

15. Add 300 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 2 min.

    Be careful not to contaminate the sample while removing the spin-column from the receiver tube. Check, whether the membrane of the spin column is completely dry. If not, pour off any remaining supernatant and place back spin-column into the receiver tube. Spin down for one additional min.



16. Place spin-column into new receiver tube (1.5-2 ml) and add 50-80 µl of **Elution** buffer to elute bound DNA.

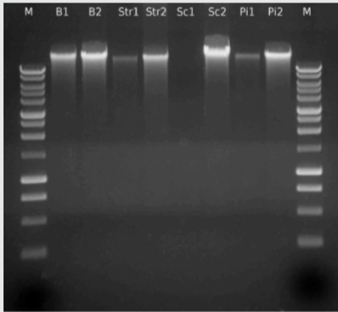
    Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transferring traces of DNA between the spin-columns do not touch the spin-column walls with the micro-pipette.

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

18. Centrifuge for 2 min at 11 000 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).

Comparison of genomic DNA purification from refractory microorganisms following either non-mechanical lysis or mechanical cell lysis using the GeneMATRIX Gram Plus & Yeast Genomic DNA Purification Kit



1 isolation without mechanical lysis and without specific enzymes;

2 isolation according to the protocol and with BeadTubeDry (glass beads), without specific enzymes.

B *Bacillus subtilis*;

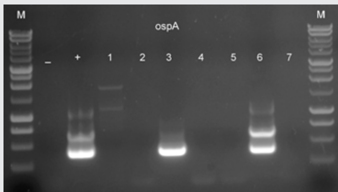
Str *Streptomyces caespitosus;*

Sc *Staphylococcus aureus*;

Pi *Pichia pastoris*;

M Perfect Plus™1 kb DNA ladder (EURx).

DNA isolation from microorganisms present in ticks



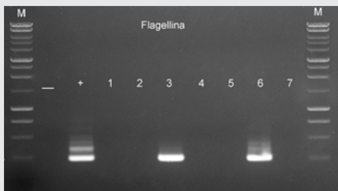
Nested PCR was performed using a DNA isolated from Ixodes ricinus as a template. Target gene was ospA on a plasmid lp54 of *Borrelia burgdorferi*. 4 µl of isolated DNA and Taq polymerase (EURx E2500) were used. Lanes:

— negative control;

+ positive control, *Borrelia burgdorferi* DNA (2 pg);

3,6 positive results;

M Perfect Plus™1 kb DNA ladder (EURx).



Nested PCR was performed using a DNA isolated from Ixodes ricinus as a template. Target gene was flagellina on *Borrelia burgdorferi* genomic DNA. 4 µl of isolated DNA and Taq polymerase (EURx E2500) were used. Lanes:

— negative control;

+ positive control, *Borrelia burgdorferi* DNA (2 pg);

3,6 positive results;

M Perfect Plus™1 kb DNA ladder (EURx).

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 plus

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 Gram Plus & Yeast Genomic DNA Purification Kit is designed for the rapid purification of genomic DNA from a wide variety of gram positive bacterial physiological groups and from a wide variety of yeast strains. The kit also allows the isolation of DNA from microorganisms present in ticks (e.g. Borrelia burgdorferi) and other small insects.***

The mechanical lysis procedure used within protocol is a universal approach for efficient cell disruption, avoiding to use expensive lytic enzymes specific to a particular group of organisms. Purified DNA is free of contaminants, such as: RNA, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others. Bacterial or yeast cells are lysed in the presence of special cell wall disintegrating buffer aided by lysozyme (bacteria). The pretreated cell wall is then effectively destroyed by the mechanical friction of glass beads with optimally adjusted diameters. Further, Proteinase K digests cellular proteins, including stripping-off DNA of all bound proteins, among them nucleases. An optimized buffer is added to provide selective conditions for DNA binding during a brief centrifugation step, 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.

- **GeneMATRIX is a 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.

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