EURx® MOLECULAR BIOLOGY PRODUCTS

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Version 1.1
September 2019

GeneMATRIX Cell Culture DNA Purification Kit

Kit for isolation of DNA from animal or human cell culture

Cat. no. E3555

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Table of Contents

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for the isolation of DNA from cultured animal or human cells.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg and maximum volume of the column reservoir is 650 µl. One minicolumn enables purification of DNA from up to 5 x 10^6 cells. For example, typical DNA yields from HeLa cells is 15–25 µg from 2 x 10^6 cells.

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature with the exception of RNase A and Proteinase K. RNase A should be kept at 2–8°C and Proteinase K at -20°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.

Content	50 preps E3555-01	150 preps E3555-02	Storage/Stability
Buffer C	1.8 ml	5.4 ml	15-25°C
Lyse C	12 ml	36 ml	15-25°C
RNase A (10 mg/ml)	0.12 ml	0.36 ml	2-8°C
Proteinase K (20 mg/ml)	0.6 ml	1.8 ml	–20°C
Sol C	12 ml	36 ml	15-25°C
Wash CX1	30 ml	90 ml	15-25°C
Wash CX2	30 ml	90 ml	15-25°C
Elution	18 ml	54 ml	15-25°C
DNA Binding Columns	50	3 x 50	15-25°C
Protocol	1	1	

Equipment and reagents to be supplied by the experimenter

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes, ethanol 96–100%, a heating block capable of incubation at 70°C.

Protocol

1. Apply 30 µl of activation **Buffer C** 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 10 min).

   - *Addition of Buffer C 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. Centrifuge the cell culture (up to 5 x 10^6 cells) in the 1.5–2 ml Eppendorf tube for 2 min at 1000 x g.

3. Carefully discard the supernatant. Add to the pellet 200 µl of **Lyse C** buffer and 2 µl of **RNase A**. Suspend the cells thoroughly by vortexing for 15 sec.

4. Incubate for 5 min at room temperature.

5. Add 10 µl of **Proteinase K** and 200 µl of **Sol C** buffer. Mix thoroughly by vortexing.

6. Incubate for 10 min at 70°C.

7. Add 200 µl of ethanol (96–100%). Mix thoroughly by vortexing.

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

9. Transfer the lysate to the **DNA binding spin-column**, placed in the collection tube.

10. Centrifuge for 1 min at 11 000 x g. Remove the spin-column, pour off supernatant and place back into the receiver tube.

    - *Continue centrifugation, if not all of the lysate passed through the column.*

11. Add 500 µl of **Wash CX1** buffer and spin down at 11 000 x g for 1 min.

12. Remove spin-column, pour off supernatant, replace back spin-column.

13. Add 500 µl of **Wash CX2** buffer and spin down at 11 000 x g for 1 min.

14. Remove spin-column, pour off supernatant, replace spin-column.

15. Spin down at 11 000 x g for 1 min to remove traces of the **Wash CX2** buffer.

16. Place spin-column into new receiver tube (1.5–2 ml) and add 50–150 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 spin-column/receiver tube assembly for 2 min at room temperature.

18. Spin down at 11 000 x g for 1 min.

19. Remove spin column, cap the receiver tube. DNA is ready for analysis/manipulations. It can be stored at 2–8°C or (preferred) at -20°C.

Safety Information

Buffer C

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 C

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 C

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 CX1

Warning

H226 Flammable liquid and vapour.
H302+**H332** Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
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.
P301+**P312** If swallowed: call a poison center/ doctor if you feel unwell.
P302+**P352** If on skin: wash with plenty of water.
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.

Wash CX2

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.

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 Cell Culture DNA Purification Kit is designed for rapid purification of total DNA (genomic, mitochondrial) from animal or human cell culture. Purified DNA is free of contaminants, such as: RNA, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others.

Cells sample is lysed in the presence of special buffer containing large amounts of chaotropic ions and Proteinase K. Proteinase K digests cellular proteins, including stripping-off DNA of all bound proteins, among them nucleases. Appropriate conditions for binding of DNA to the GeneMATRIX resin is created by addition of ethanol to the lysate. During brief centrifugation step DNA binds to the silica membrane in the spin-column, while contaminants pass through. 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.

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