GeneMATRIX Cell Culture DNA Purification Kit

Kit for isolation of DNA from animal or human cell culture

Cat. no. E3555

Version 3.1

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

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Note 1: *One minicolumn enables purification of DNA from up to 10^7 cells.*
Note 2: *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 3: *All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.*
Note 4: *The kit does not contain 96 % ethanol, the reagent required during the DNA isolation procedure. This reagent needs to be provided by the user.*

Protocol

1. *Apply 40 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.*

Note 1: *Addition of Buffer C 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. *Centrifuge the cell culture (up to 10^7 cells) in the 1.5-2 ml Eppendorf tube for 2 min at 5000 rpm.*

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* **96 % ethanol**.

8. *Mix thoroughly by vortexing.*

9. *Centrifuge for 1 min at 12000 rpm.*

10. *Transfer the lysate to the spin-column, placed in the collection tube.*

11. *Centrifuge for 1 min at 12000 rpm.*

Note 1: *Continue centrifugation, if not all of the lysate passed through the column.*

12. *Take out spin-column, discard flow-through and place back spin-column in the collection tube.*

13. *Add 500 µl of* **Wash CX1** *buffer to the spin-column and centrifuge for 1 min at 12000 rpm.*

14. *Take out spin-column, discard flow-through and place back spin-column in the collection tube.*

15. *Add 500 µl of* **Wash CX2** *buffer to the spin-column and centrifuge for 2 min at 12000 rpm.*

16. *Place the spin-column in a new collection tube (1.5-2 ml) and add 100-200 µl of* **Elution** *buffer (10 mM Tris-HCl, pH 8.5) heated to 70° C to elute bound DNA.*

Note 1: *Addition of eluting buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.*

Note 2: *The following eluting solutions can be used:*

1. *5-10 mM Tris-HCl buffer, pH 8.0-9.0*
2. *0.5-1 x TE buffer, pH 8.0-9.0 (not recommended for DNA sequencing).*
3. *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 8.0-9.0.*

17. *Incubate the spin-column/collection tube assembly for 3 min at room temperature.*

18. *Centrifuge the spin-column for 1 min at 12000 rpm.*

Optional:

19. *Repeat elution once again as described in steps 16-18.*

Note 1: *This step improves DNA recovery from the column. A new collection tube can be used to prevent dilution of the first eluate or collection tube from step 16 can be reused to combine the eluates.*

Note 2: *More than 200 µl should not be used to elute into a single 1.5 ml microcentrifuge tube, as the spin-column will come into contact with the eluate, causing DNA contamination.*

20. *Discard spin-column, cap the collection tube. DNA is ready for analysis/manipulation. It can be stored either at 2÷8° C or at -20° C.*

__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.