EURx Molecular Biology Products

GeneMATRIX Agarose-Out DNA Purification Kit

Universal kit for purification of DNA from agarose gels

Cat. no. 3540

Version 6.1

January, 2008

Distributor:

roboklon

Roboklon GmbH
Kantstrasse 65, 10627 Berlin
phone 030 318 09 372 fax 030 310 19 197

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

EURx Ltd. 80-297 Gdansk Poland ul. Przyrodnikow 3, NIP 957-07-05-191, KRS 0000202039,
www.eurx.com.pl orders: *email*: orders@eurx.com.pl *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Note 1: Once the kit is unpacked, store components at room temperature. In case of occasional buffer ingredients precipitation, simply warm up in 37°C water bath, until clarified. Buffers Orange-A and Wash-A1 should be stored in the dark and cold, prefereably at 4-15°C.

Note 2: To obtain optimal results of automated DNA sequencing it is recommended to use 0.3-0.6 pmols of DNA template per reaction (examplified by 200-400 ng of 1 kb DNA fragment) isolated with GeneMatrix Agarose-Out DNA Purification Kit.

Note 3: Buffers: Orange-A and Wash-A1 may form reactive and toxic compounds when combined with acids. Do not add bleach or acidic solutions to the sample preparation waste.

Note 4: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

Note 5: The kit is designed to isolate DNA molecules, ranging in size from approximately 100 bp to 10 kb, from TAE- or TBE-agarose gels. It is also possible to purify DNA fragments up to 20 kb or more, with decreased isolation yields.

Protocol

1. Apply 40 µl of activation Buffer A onto the spin-column (do not spin) and keep it at room temperature till transfering dissolved agarose solution to the spin-column.

   Note 1: Addition of Buffer A 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. Cut out DNA from agarose gel. Weight the agarose piece; the weight should not exceed 250 mg. Place the agarose slice(s) into Eppendorf tube.

   Note 1: Avoid excess of agarose cut out along with DNA fragment.

   Note 2: If weight of the gel slice exceeds 250 mg, use more than one column.

   Note 3: It is highly recommended that electrophoresis buffer is not re-used, due to pH changes which negatively affect DNA isolation from a gel.

3. Add 600 µl of orange-coloured Orange-A buffer. Mix by three-fold inverting.

4. Incubate in heating block or water bath at 55°C, mixing every 1-2 minutes by two-fold inverting, until agarose will dissolve completely. The appearance of the uniform, orange-coloured solution indicates completion of the process.

   Note 1: Agarose slice(s) will dissolve within 5 to 10 min, depending on used agarose gel concentration and weight of agarose piece.

5. Pour dissolved agarose solution into spin-column/receiver tube assembly.

6. Spin down in a microcentrifuge at 12,000 rpm for 1 minute.

7. Remove spin column, pour off the supernatant, replace back the spin-column and place into a microcentrifuge.

8. Add 500 µl of Wash-A1 buffer and spin down at 12,000 rpm for 1 minute.

9. Remove spin column, pour off supernatant, replace back the spin-column.

10. Add 650 µl of Wash-AX2 buffer and spin down at 12,000 rpm for 1 minute.

11. Remove spin column, pour off supernatant, replace back the spin-column.

12. Spin down at 12,000 rpm for 2 minutes to remove traces of Wash-AX2 buffer.

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

Note 1: Addition of eluting buffer directly onto the center of the membrane improves DNA yield.
Note 2: To improve recovery of larger DNA fragments (above 5 kb) it is recommended to elute with buffer heated to 80°C.
Note 3: For elution of DNA the Elution-A buffer is highly recommended. The buffer is prepared using ultrapure water with trace addition of buffering compounds. The Elution-A buffer will not interfere with subsequent DNA manipulations, such as DNA sequencing, ligation or restriction digestion, among others.
Note 4: It is possible to reduce the volume of eluting buffer below 50 µl (no less than 20 µl). However, recovery of DNA will gradually decrease.
Note 5: To obtain optimal results of automated DNA sequencing it is recommended to use 0.3-0.6 pmols of DNA template per reaction (examplified by 200-400 ng of 1 kb DNA fragment) isolated with GeneMatrix Agarose-Out DNA Purification Kit.

14. Incubate spin-column/receiver tube assembly for 2 minutes at room temperature.

15. Spin down at 12,000 rpm for 1 minute.

16. Remove spin column, cap the receiver tube. Isolated DNA is ready for analysis/manipulations. It can be stored at 4°C or (preferred) 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 Agarose-Out DNA Purification Kit is designed to isolate ultrapure linear or circular DNA molecules, ranging in size from approximately 100 bp to 10 kb, from TAE- or TBE-agarose gels. It is also possible to purify DNA fragments up to 20 kb or more, with gradually decreasing yields. Coloured solubilizing buffer helps both in monitoring agarose dissolving and in simultaneous processing of multiple samples. Besides agarose many other contaminants are effectively removed: ethidium bromide, RNA, primers, enzymes and other proteins, lipids, endotoxins, dyes, detergents, nucleotides, radio- and chemical labels, EDTA, problematic restriction and ligation inhibitors, buffers and salts. Optimized buffer is added to provide selective conditions for DNA binding during brief centrifugation, while contaminants pass through the GeneMATRIX in the spin-column. Traces of solubilized agarose and other contaminants remaining on the membrane are efficiently removed in two wash steps. The membrane used is particularly designed toward removal of problematic inhibitors of restriction and ligation of DNA. High-quality DNA is then eluted in low salt buffer, e.g.: Tris-HCl, TE or water. Izolated DNA is ready for downstream applications without the need for ethanol precipitation.

EURx Distributor:
Roboklon GmbH
Kantstrasse 65, 10627 Berlin
phone 030 318 09 372 fax 030 310 19 197