EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Agarose-Out DNA Purification Kit

Universal kit for purification of DNA from agarose gels

Cat. no. E3540

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Table of Contents

Content	50 preps E3540-01	150 preps E3540-02	Storage/Stability
Buffer A	1.8 ml	5.4 ml	15-25°C
Orange A	39 ml	117 ml	15-25°C
Wash A1	30 ml	90 ml	15-25°C
Wash AX2	36 ml	108 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	

Introductory Notes

NOTE 1 · Kit Specification. 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.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 20 µg. Up to 250 mg agarose can be processed per spin column. The maximum volume of the column reservoir is 650 µl. 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 · Kit Compounds Storage. 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.

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. 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.

The kit provides spin columns and buffers for silica-membrane-based purification of DNA fragments from gels (up to 250 mg slices). Purified DNA can be used in routine molecular biology applications such as PCR, sequencing and cloning. Protocol offers a simple bind-wash-elute procedure. Gel slices are dissolved in a colored buffer, allowing monitoring of complete solution mixing and makes purification procedure more reproducible. The mixture is then applied to the minicolumn where nucleic acids adsorbs to the silica membrane in the high-salt conditions provided by the buffer. Impurities are washed away and pure DNA is eluted in a small volume of elution buffer.

Equipment and reagents to be supplied by the experimenter

1. Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes, scalpel, heating block or water bath set at 55°C.

Protocol

Part I Solubilization of agarose gel

1. Apply 30 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.

   - *Addition of Buffer A 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.*

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.

   - *Avoid excess of agarose cut out along with DNA fragment.*

   - *If weight of the gel slice exceeds 250 mg, use more than one column.*

   - *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 2.5 volumes of orange-coloured **Orange A** buffer to 1 volume of gel (100 mg ~ 100 µl). Mix by three-fold inverting.

   - *For example, add 250 µl of Orange A buffer to each 100 mg of gel.*

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

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

Part II Adsorption to the membrane, washing and elution of DNA fragments

1. Apply up to 600 µl of a dissolved agarose solution 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 into the receiver tube.
2. 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.
3. Add 500 µl of **Wash A1** buffer and spin down at 11 000 x g for 1 min.
4. Remove spin-column, pour off supernatant, replace back spin-column.
5. Add 600 µl of **Wash AX2** buffer and spin down at 11 000 x g for 1 min.
6. Remove spin-column, pour off supernatant, replace spin-column.
7. Spin down at 11 000 x g for 1 min to remove traces of the **Wash AX2** buffer.
8. Place spin-column into new receiver tube (1.5–2 ml). Add 50–80 µl of **Elution** buffer to elute bound DNA.

   - *Addition of eluting buffer directly onto the center of the membrane improves DNA yield.*
   - *To improve recovery of larger DNA fragments (above 5 kb) it is recommended to elute with buffer heated to 80°C.*
   - *For elution of DNA the Elution buffer is highly recommended. The buffer is prepared using ultrapure water with trace addition of buffering compounds. The Elution buffer will not interfere with subsequent DNA manipulations, such as DNA sequencing, ligation or restriction digestion, among others.*
   - *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.*

9. Incubate spin-column/receiver tube assembly for 1 min at room temperature.
10. Spin down at 11 000 x g for 1 min.
11. Remove spin column, cap the receiver tube. Isolated DNA is ready for analysis/manipulations. It can be stored at 2–8°C or (preferred) at -20°C.

Safety Information

Buffer A

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.

Orange A

Warning

H302+**H332** Harmful if swallowed or if inhaled.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
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.
EUH032 Contact with acids liberates very toxic gas.

Wash A1

Warning

H302+**H332** Harmful if swallowed or if inhaled.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
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.
EUH032 Contact with acids liberates very toxic gas.

Wash AX2

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 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.

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. Isolated DNA is ready for downstream applications without the need for ethanol precipitation.

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