EURx Molecular Biology Products

GeneMATRIX Basic DNA Purification Kit

Universal kit for purification of PCR products / DNA after enzymatic reactions, isolation of DNA from agarose gels and isolation of plasmid DNA from bacteria.

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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, except buffer **Cell R**, which should be stored at 2÷8°C. This will ensure the best performance, due to preserving activity of RNaseA included into the buffer. In case of occasional buffer ingredients precipitation, simply warm up in 37°C water bath, until clarified.

Note 2: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

Note 3: Buffer **Basic** may form reactive and toxic compounds when combined with acids. Do not add bleach or acidic solutions to the sample preparation waste.

Note 4: **PCR/DNA Clean-up Protocol** removes primers below 40 nt and double-stranded DNA below 20 bp. However, common short by-products of not optimal or problematic PCR, known as primer-dimers, also consist of double-stranded DNA. They are produced from self-annealed and extended primers and co-migrate on a gel along with unincorporated single-stranded DNA primers. These double-stranded DNA artefacts co-purify with an expected PCR product, if their length exceeds 20 bp. If the removal of primer-dimers is necessary, we recommend PCR reaction optimization and/or agarose gel electrophoresis followed by isolation of PCR product using **Agarose-Out Protocol**.

Note 5: **Agarose-Out Protocol** 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 6: **Plasmid DNA Purification Protocol** is designed to isolate high purity plasmid DNA from various species of bacteria, including recombinant *Escherichia coli* strains. Recommended culture volume is 1.0-3 ml.

Note 7: Maximum binding capacity 20 µg.

Equipment and reagents to be supplied by user:

1. For all protocols: microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5-2 ml tubes.
2. For Agarose-Out protocol – equipment necessary for visualization and excision of DNA band. In most cases: UV lamp and the scalpel.

Protocol I

PCR / DNA Clean-Up

The Protocol allows for quick purification of PCR products, restriction fragments, DNA molecules after enzymatic treatment and chemical or isotopic labeling.

1. Apply 40 µl of activation **Buffer Uni** onto the spin-column (do not spin) and keep it at room temperature till transfering mixture (p. 3) to the spin-column.

   Note 1: Addition of Buffer Uni 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. Add 400 µl of **Basic** buffer to DNA sample and mix.

   Note 1: Maximum volume of a DNA sample can not exceed 200 µl.

3. Pour the mixture into spin-column/receiver tube assembly.

4. Spin down in a microcentrifuge at 11 000 x g for 1 minute.

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

6. Add 500 µl of **Wash UX1** buffer and spin down at 11 000 x g for 1 minute.

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

8. Add 650 µl of **Wash UX2** buffer and spin down at 11 000 x g for 1 minute.

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

10. Spin down at 11 000 x g for 2 minutes to remove traces of **Wash UX2** buffer.

11. Place spin-column into new receiver tube (1.5-2 ml). Add 50-150 µl of **Elution** 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 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.
    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.

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

13. Spin down at 11 000 x g for 1 minute.

14. 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.

Protocol II

Agarose-Out

Protocol 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.

1. Apply 40 µl of activation **Buffer Uni** 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 Uni 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 **Basic** 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.

   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 11 000 x g for 1 minute.

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

8. Add 400 µl of **Basic** buffer and spin down at 11 000 x g for 1 minute.

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

10. Add 650 µl of **Wash UX2** buffer and spin down at 11 000 x g for 1 minute.

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

12. Spin down at 11 000 x g for 2 minutes to remove traces of **Wash UX2** buffer.

13. Place spin-column into new receiver tube (1.5-2 ml) and add 50-80 µl of **Elution** 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 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.
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).

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

15. Spin down at 11 000 x g for 1 minute.

16. 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.

Protocol III

Plasmid Isolation Protocol

The Protocol allows for quick isolation of plasmid DNA from bacterial cultures.

1. Apply 40 µl of activation **Buffer Uni** 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 Uni 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. Pour cells from overnight culture (11-14 h) into 1.5-2 ml tubes. Spin down 1.0-3 ml in a microcentrifuge at 12 000 x g for 2 minutes. Pour off the supernatant and blot tubes upside-down on paper towel to remove any remaining media.

   Note 1: E.coli strains recommended for plasmid isolation have $endA^-$ genotype, such as: DH5a, DH1, JM103-109, XL1-Blue, MM294 and C600. While $endA^+$ strains, such as BL21, RR1, DH11S, JM101, HB101,TG1 and TB1 can also be used, but they yield lower quality DNA.

3. Add 250 µl of **Cell R** buffer and completely resuspend the cell pellet.

4. Add 200 µl of blue-coloured **Lysis Blue** buffer. Mix gently, but completely by several-fold inverting, until uniform blue colour of cell resuspension is obtained.

   Note 1: Alkaline Lysis Blue buffer contains SDS, which can precipitate at temperatures below 20°C. In this case warm the buffer up in 37°C water bath, until clarified.
   Note 2: Forceful mixing should be avoided, as it can cause irreversible denaturation of plasmid DNA molecules as well as contamination with genomic DNA fragments.

5. Add 350 µl of neutralization and binding buffer **Neutral B**. Mix by several-fold inverting, until blue colour will disappear.

6. Spin down in a microcentrifuge at 12 000 x g for 7 minutes.

7. Pour the supernatant from step 5 into the spin-column placed in the receiver tube.

8. Spin down at 11 000 x g for 1 minute.

9. Remove the spin-column, pour off supernatant and place back into the receiver tube.

10. Add 500 µl of **Wash UX1** buffer and spin down at 11 000 x g for 1 minute.

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

12. Add 650 µl of **Wash UX2** buffer and spin down at 11 000 x g for 1 minute.

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

14. Spin down at 11 000 x g for 2 minutes to remove traces of the **Wash UX2** buffer.

15. Place spin-column into new receiver tube (1.5-2 ml). Add 50-100 µl of **Elution** 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 plasmids (above 6 kb) it is recommended to elute with buffer heated to 80°C.
    Note 3: 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.
    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.

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

17. Spin down at 11 000 x g for 2 minutes.

18. Remove spin-column, cap the receiver tube. Plasmid DNA is ready for analysis/manipulations. It can be stored either at 2÷8°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 Basic DNA Purification Kit is a useful basic tool in any laboratory working with DNA. It allows to perform three basic laboratory techniques: DNA purification after enzymatic treatment, isolation of DNA from agarose gels and the isolation of plasmid DNA from bacterial cultures. Combining these capabilities kit allows to streamlin work and minimizing the costs of research projects.

PCR / DNA Clean-Up Protocol allows for purification of DNA fragments, which were subjected to or obtained as a result of various modifications and reactions: PCR products, restriction digests, after kinasing, dephosphorylation, end-trimming/repair, ligation, enzymatic or chemical modification, among others. Fragment of sizes from approximately 100 bp to over 15 kb can be obtained in ultrapure form. Effectively removed are contaminants such as: ethidum bromide, primers (below 40 nt), short double-stranded DNA (below 20 bp), RNA, *Taq* DNA Polymerase, *Pfu* DNA Polymerase, endo- and exonucleases, DNA-binding and modifying proteins, BSA and other enzymes/proteins, lipids, endotoxins, dyes, detergents, nucleotides, radio- and chemical labels, EDTA, problematic restriction and ligation inhibitors, buffers and salts.

Agarose-Out Protocol is designed to isolate 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. 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.

Plasmid Isolation Protocol allows for purification of high purity plasmid DNA from various species of bacteria, including recombinant *Escherichia coli* strains. Plasmid DNA contaminants such as: RNA, single-stranded DNA, enzymes/proteins, lipids, dyes, detergents, nucleotides, EDTA, problematic restriction and ligation inhibitors, buffers and salts are effectively removed from crude bacterial lysate. Coloured lysis buffer helps both in monitoring cell solubilization progress as well as simultaneous processing of multiple samples.

For all protocols optimized buffer is added to provide selective conditions for DNA binding to the **GeneMATRIX** membranes during brief centrifugation, while contaminants pass through the spin-column. Traces of contaminants remaining on the membrane are efficiently removed in two wash steps. 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.