

GeneMATRIX PCR / DNA Clean-Up Purification Kit

Kit for purification of PCR products / DNA after enzymatic reactions

Cat. no. E3520

Version 3.1

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

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.

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

Note 4: This kit selectively 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 our GeneMATRIX Agarose-Out Purification Kit.

Note 5: Maximum binding capacity 20 µg.

Protocol

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

Note 1: Addition of Buffer DX 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 orange-coloured **Orange DX** buffer to DNA sample and mix.

Note 1: Maximum volume of a DNA sample can not exceed 250 µl.

3. Pour the mixture into spin-column/receiver tube assembly.
4. Spin down in a microcentrifuge at 12,000 rpm 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 DX1** buffer and spin down at 12,000 rpm for 1 minute.
7. Remove spin column, pour the supernatant off, replace back the spin-column.
8. Add 650 µl of **Wash DX2** buffer and spin down at 12,000 rpm for 1 minute.
9. Remove spin column, pour the supernatant off, replace back the spin-column.
10. Spin down at 12,000 rpm for 2 minutes to remove traces of **Wash DX2** buffer.

11. Place spin-column into new receiver tube (1.5-2 ml). Add 50-150 μ l 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 μ l (no less than 20 μ l). However, recovery of DNA will gradually decrease.

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

13. Spin down at 12,000 rpm 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.

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, hybridization among others. Additional advantage is reproducibility of matrix performance, as component preparation is carried at Eurx Ltd.

GeneMATRIX PCR / DNA Clean-Up Purification Kit is designed to isolate 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: ethidium 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. **GeneMatrix** is especially optimized toward binding DNA molecules over the very wide range of sizes: from 100 bp to over 15 kb as well as toward removal of problematic inhibitors of restriction and ligation of DNA. Coloured binding buffer is very helpful in simultaneous processing of multiple samples. 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.