EURx® MOLECULAR BIOLOGY PRODUCTS

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Version 2.1
September 2019

GeneMATRIX Short DNA Clean-Up Purification Kit

Kit for purification of short single-stranded and double-stranded DNA fragments after enzymatic reactions

Cat. no. E3515

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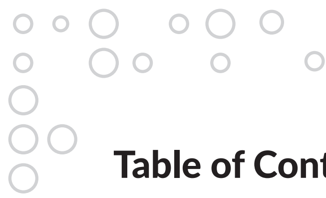

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Content	25 preps E3515-01	100 preps E3515-02	Storage/Stability
Buffer SF	0.9 ml	3.6 ml	15-25°C
SFB	21 ml	84 ml	15-25°C
Wash SFX	27 ml	108 ml	15-25°C
Elution	3 ml	12 ml	15-25°C
DNA Binding Columns	25	2 x 50	15-25°C
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Introductory Notes

NOTE 1 · Kit Specification. This kit removes unincorporated nucleotides, enzymes, salts and others. Procedure do not removes short by-products of not optimal or problematic PCR, known as primer-dimers and unused in PCR reaction primers. This protocol is designed for cleanup of oligonucleotides and double-stranded DNA from 10 to 10 000 base par.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg. The maximum volume of the column reservoir is 650 µl.

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.

The kit provides spin columns and buffers for silica-membrane-based purification of DNA fragments (especially short) from PCR and others enzymatic reactions. Purified DNA can be used in routine molecular biology applications such as PCR or sequencing. Protocol offers a simple bind-wash-elute procedure. Procedure removes nucleotides, enzymes, mineral oil, salts, and other impurities from DNA samples. Binding buffer is added directly to the PCR sample or other enzymatic reaction, and 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.

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes.
- Ethanol (96-100%) or isopropanol.

Protocol

1. Apply 30 µl of activation **Buffer SF** onto the spin-column (do not spin) and keep it at room temperature till transfering mixture (point 4) to the spin-column (for best results at least 10 min).

   Addition of Buffer SF 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. Add 5 volumes of **SFB** buffer to 1 volume of DNA sample and mix well.

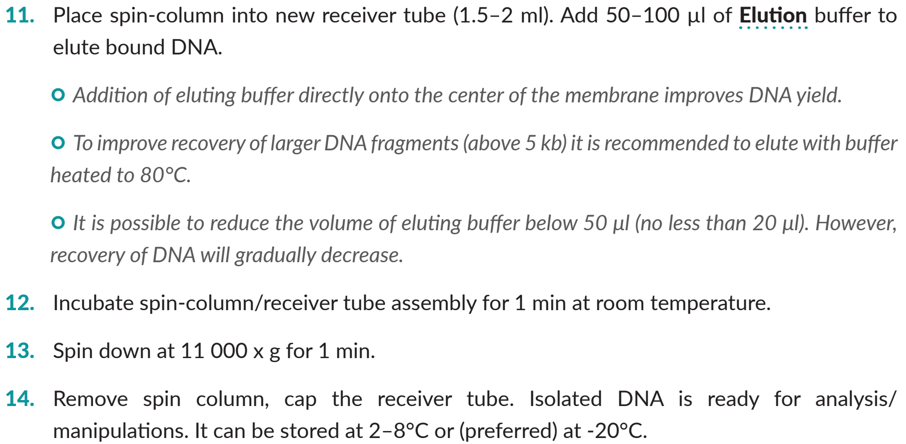
   For example, add 200 µl SFB buffer to a 40 µl reaction sample.

   The minimum volume of reaction is 40 µl. When the volume of reaction is below 40 µl add ddH_2O to 40 µl. The maximum volume of reaction is 120 µl. In this case apply the mixture into spin column in portions of approximately 650 µl.

3. Add 1.2 volumes of ethanol (96-100%) (or 1.0 volume of isopropanol) to 1 volume of DNA mixture and mix well.

   For example, add 288 µl of ethanol (or 240 µl isopropanol) to a 240 µl mixture from point 2.

4. Apply up to 650 µl of the mixture 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.
5. 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.
6. Add 450 µl of **Wash SFX** buffer and spin down at 11 000 x g for 1 min.
7. Remove spin-column, pour off supernatant, replace back spin-column.
8. Add 450 µl of **Wash SFX** buffer and spin down at 11 000 x g for 1 min.
9. Remove spin-column, pour off supernatant, replace spin-column.
10. Spin down at 11 000 x g for 1 min to remove traces of the **Wash SFX** buffer.



11. Place spin-column into new receiver tube (1.5–2 ml). Add 50–100 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.*
- *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 1 min at room temperature.

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

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.

Safety Information

Buffer SF

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.

Wash SFX

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.

SFB

Danger

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317 May cause an allergic skin reaction.
P261 Avoid breathing vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P284 [In case of inadequate ventilation] wear respiratory protection.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+**P313** If eye irritation persists: get medical advice/ attention.
P342+**P311** If experiencing respiratory symptoms: call a poison center or doctor/ physician.
P333+**P313** If skin irritation or rash occurs: get medical advice/attention.
P302+**P352** If on skin: wash with plenty of water.

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 Short 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.

This kit is especially optimized toward binding oligonucleotides and ds DNA molecules over the range of sizes: from 10 b to 10 kb as well as toward removal of problematic inhibitors of restriction and ligation of DNA. Effectively removed are contaminants such as: nucleotides, ethidum bromide, Taq DNA Polymerase, Pfu DNA Polymerase, endo- and exonucleases, DNA-binding and modifying proteins, BSA and other enzymes/proteins, lipids, dyes, detergents, radio- and chemical labels, EDTA, problematic restriction and ligation inhibitors, buffers and salts. Sample is applied to a DNA binding spin column where DNA molecules are adsorbed to the matrix and contaminants are efficiently washed away. 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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