EURx® MOLECULAR BIOLOGY PRODUCTS

Version 1.4
May 2025

GeneMATRIX Plasmid Miniprep DNA Purification Kit

Kit for isolation of high-purity plasmid DNA (1.0–3.0 ml bacterial culture)

Cat. no. E3500

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

Table of Contents

Content	50 preps E3500-01	150 preps E3500-02	Storage/Stability
Buffer PL	1.8 ml	5.4 ml	15-25°C
Cell R *	15ml	45 ml	2-8°C
Lysis Blue	15 ml	45 ml	15-25°C
Neutral B	21 ml	63 ml	15-25°C
Wash PLX1	30 ml	90 ml	15-25°C
Wash PLX2	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	

* Contains RNase A (100 µg/ml).

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for the rapid isolation of very high purity plasmid DNA from various species of Gram- bacteria, including recombinant *Escherichia coli* strains.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg. Use either early stationary phase or log phase bacterial cultures for obtaining highest DNA quality. Due to the differences in growth characteristics of bacteria species, it is recommended to perform a preliminary experiment for determining the optimal amount of bacterial culture per prep. In general, the weight of the cell pellet should not exceed 50 mg per single minicolumn and the volume of the culture volume should not exceed 3.0 ml per single minicolumn. In case of minicolumn clogging due to high lysate viscosity, reduce the initial amount of bacteria used for isolation.

NOTE 3 · Kit Compounds Storage. 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 RNase A included into the buffer. 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 concentration changes of buffer components. To obtain high quality DNA, stick carefully to the protocol provided below.

NOTE 5 · Elution buffer is a low salt solution, that contains no metal ion chealators (e.g. EDTA) that can inhibit subsequent enzymatic reactions. Elution buffer composition is suitable for downstream applications such as digestion with restriction enzymes, phosphorylation, ligation, Sanger sequencing, NGS etc. It is also possible to elute the DNA with Tris-HCl, water or TE.

The kit enables purification of up to 25 µg molecular biology grade plasmid DNA for use in routine molecular biology applications such as PCR, sequencing and cloning. Protocol offers a simple bind-wash-elute procedure. First, bacterial cultures are lysed and after neutralization the lysates are cleared by centrifugation. The cleared lysates are then applied to the minicolumn where plasmid DNA adsorbs to the silica membrane. Impurities are washed away and pure DNA is eluted in a small volume of elution buffer.

Equipment and reagents to be supplied by the user

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes.

Protocol

Part I Preparation and cell lysis.

1. Apply 30 µl of activation **Buffer PL** onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

   - *Addition of Buffer PL 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. Pellet 1-3 ml of the overnight culture in 1.5-2 ml Eppendorf tubes by centrifuging at 12 000 x g for 2 min. Pour off the supernatant and blot tubes upside-down on paper towel to remove any remaining media.

   - *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 250 µl of blue-coloured **Lysis Blue** buffer. Mix gently by several-fold inverting, until uniform blue colour of cell lysate is visible.

   - *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.*

   - *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 disappear.

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

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

1. Apply up to 600 µl of a clear supernatant 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 supernatant to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, discard the flow-through and place back into the collection tube.
3. Add 500 µl of **Wash PLX1** buffer and spin down at 11 000 x g for 1 min.
4. Remove the spin-column, discard the flow-through and place back into the collection tube.
5. Add 600 µl of **Wash PLX2** buffer and spin down at 11 000 x g for 1 min.
6. Remove the spin-column, discard the flow-through and place back into the collection tube.
7. Spin down at 11 000 x g for 1 min to remove traces of the **Wash PLX2** buffer.
8. Place spin-column into new receiver tube (1.5–2 ml). Add 50–100 µl of **Elution** buffer to elute bound plasmid DNA.

   - *Addition of eluting buffer directly onto the center of the membrane improves DNA yield.*
   - *To improve recovery of larger plasmids (above 6 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.

   - *For larger plasmids (above 6 kb) it is recommended to prolong the incubation time to 10 min.*
10. Spin down at 11 000 x g for 1 min.
11. 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.

Safety Information

Buffer PL

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.

Lysis Blue

Warning

H315 Causes skin irritation.
H319 Causes serious eye irritation.
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.
P337+P313 If eye irritation persists: get medical advice/ attention.
P302+P352 If on skin: wash with plenty of water.
P332+P313 If skin irritation occurs: get medical advice/attention.

Wash PLX2

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.

Neutral B

Danger

H302+H332 Harmful if swallowed or if inhaled.
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.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P284 [In case of inadequate ventilation] wear respiratory protection.
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.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333+P313 If skin irritation or rash occurs: get medical advice/attention.

Wash PLX1

Warning

H226 Flammable liquid and vapour.
H302+H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
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.
P301+P312 If swallowed: call a poison center/ doctor if you feel unwell.
P302+P352 If on skin: wash with plenty of water.
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.

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 Plasmid Miniprep DNA Purification Kit is designed to isolate very 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. The membranes used for construction of the Plasmid Miniprep Kit is especially optimized toward removal of problematic inhibitors of restriction of DNA as well as non-specific endo- and exonucleases. Coloured lysis buffer helps both in monitoring cell solubilization progress as well as simultaneous processing of multiple samples. Optimized buffer is added to provide selective conditions for DNA binding to the GeneMATRIX 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.

EURx®
MOLECULAR
BIOLOGY
PRODUCTS

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