EURx® MOLECULAR BIOLOGY PRODUCTS

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Version 1.1
September 2019

GeneMATRIX Agrobacterium Plasmid Miniprep DNA Purification Kit

Kit for isolation of high-purity plasmid DNA
from Agrobacterium tumefaciens (0.5–2 ml bacterial culture)

Cat. no. E3535

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Content	50 preps E3535-01	150 preps E3535-02	Storage/Stability
Buffer AG	1.8 ml	5.4 ml	15-25°C
Cell R *	9 ml	27 ml	2-8°C
LBG	9 ml	27 ml	15-25°C
BL **	1.2 ml	3.6 ml	–20°C
Lysis Blue	18 ml	54 ml	15-25°C
Neutral AG	15 ml	45 ml	15-25°C
Binding Buffer	18 ml	54 ml	15-25°C
Wash AGX1	30 ml	90 ml	15-25°C
Wash AGX2	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).

** Contains lysozyme (20 mg/ml).

Equipment and reagents to be supplied by the experimenter

- Ethanol [96–100% v/v], microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml collection tubes. Equipment needed to heat the sample to 37°C – heating block or laboratory incubator. Ice or refrigerator to perform precipitation on 4°C.

Introductory Notes

NOTE 1 · Kit Specification. The Agrobacterium Plasmid Miniprep DNA Purification Kit is designed for purification of large plasmids DNA from soil bacteria Agrobacterium tumefaciens which were cultured in liquid media.

NOTE 2 · Maximum Sample Portion. One preparation enables purification of DNA from up to 2 ml bacterial liquid culture. 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, with the exception of CellR buffer and BL buffer. CellR buffer should be kept at 2–8°C and BL at -20°C. This will ensure the best performance, due to preserving activity of RNaseA included into the CellR buffer and lysozyme in BL buffer. In case of occasional buffer ingredients precipitation (especially in LyseBlue buffer), 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 components concentration changes. To obtain high quality DNA, stick carefully to the protocol provided below.

Protocol

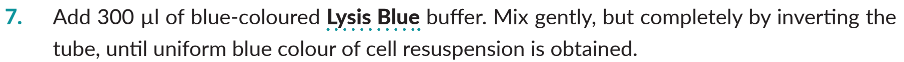
1. Apply 30 µl of activation **Buffer AG** onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

   - *Addition of Buffer AG 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. Pour cells from 0.5–2 ml liquid culture into 1.5–2 ml tubes. Spin down in a microcentrifuge at 11 000 x g for 2 min. Pour off the supernatant and blot tubes upside-down on paper towel to remove any remaining media.
3. Add 150 µl of **Cell R** buffer and completely resuspend the cell pellet.
4. Add 150 µl of **LBG** buffer and mix well by pippeting.
5. Add 20 µl of **BL** buffer and mix well by pippeting.
6. Incubate the sample at 37°C for 15 min.

   - *Incubate the sample until the solution becomes clear and lysis is complete.*



7. Add 300 µl of blue-coloured **Lysis Blue** buffer. Mix gently, but completely by inverting the tube, until uniform blue colour of cell resuspension is obtained.

   - *Forceful mixing should be avoided, as it can cause irreversible denaturation of plasmid DNA molecules as well as contamination with genomic DNA fragments. But mixing should be long enough to thoroughly combine components and the mixture should be uniform and homogenous.*

   - *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.*

8. Add 250 µl of neutralization buffer **Neutral AG**. Mix by several-fold inverting, until blue colour will disappear. Incubate on ice for 10 min.
9. Spin down in a microcentrifuge at maximum speed for 7 min.
10. Transfer the supernatant to a new 2 ml tube. Add 300 µl **Binding Buffer** to the sample, and mix thoroughly by vortexing or pippeting. Do not centrifuge.
11. Add 100 µl ethanol (96–100%) to the mixture, and mix thoroughly by vortexing or pippeting.
12. Apply up to 600 µl of a 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.
13. 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.
14. Add 500 µl of **Wash AGX1** buffer and spin down at 11 000 x g for 1 min.
15. Remove spin-column, pour off supernatant, replace back spin-column.
16. Add 600 µl of **Wash AGX2** buffer and spin down at 11 000 x g for 1 min.
17. Remove spin-column, pour off supernatant, replace spin-column.
18. Spin down at 11 000 x g for 1 min to remove traces of the **Wash AGX2** buffer.
19. 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 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.*

20. Incubate spin-column/receiver tube assembly for 2 min at room temperature.
21. Spin down at 11 000 x g for 1 min.
22. 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 AG

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.

LBG

Warning

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.

Lysis Blue / Neutral AG

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.

Binding Buffer

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 AGX1

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.

Wash AGX2

Warning

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 synonymous for a family of synthetic, new generation, nucleic acid binding membranes.

The GeneMATRIX membrane family has gained fame for two striking features: First, for their extraordinary high binding capacity, allowing to isolate nucleic acids with optimal yield. Second, for their remarkably high specificity. Even compounds of pronounced chemical similarity such as DNA, RNA and polysaccharides are easily differentiated amongst each other by the selectivity of these highly optimized matrices. This feature allows to isolate highly pure nucleic acids, that remain to work reliable even after being subjected to extended storage periods (years). Or, directly upon isolation, when 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, etc ...

We take great care to fine-tune all components and variables of the nucleic acid purification system towards each other – a multi-parameter optimization. All matrices ship conveniently pre-packed in a ready-to-use spin-column format. Spin columns are specifically constructed for precise adjustment of liquid flow-through rate to optimal values. Novel binding and washing buffers are developed to take full advantage of GeneMATRIX unique capacity, resulting in isolation of biologically active, high-quality nucleic acids. And, last not least, a lot of time and efforts went into development of the various GeneMATRIces, thus providing a platform of unique chemical composition.

High and continuous reproducibility of matrix performance is always warranted, since component preparation as well as stringent quality control is entirely performed in-house at EURx Ltd.

Whatever your experience with nucleic acids isolation kits may look like, most likely you will encounter a difference with GeneMATRIX. And, we are so much convinced, you'll love it. Enjoy.

GeneMATRIX Agrobacterium Plasmid Miniprep DNA Purification Kit is designed to isolate high purity plasmid DNA from soil bacteria Agrobacterium tumefaciens. 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 procedure is especially optimized toward isolation of palsmids larger than 10 kb from Agrobacterium species. The addition of the lysozyme incubation (with BL buffer) improves lysis and increases plasmid yield. 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.

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