EURx® MOLECULAR BIOLOGY PRODUCTS

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# AnEx Plasmid Midiprep DNA Purification Kit

Kit for isolation of ultrapure plasmid DNA in anion-exchange technology.

**Cat. no. E3790**

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# Table of Contents



| Content | 4 preps E3790-01 | 8 preps E3790-02 | Storage/Stability |
|---|---|---|---|
| Cell Res | 30 ml | 60 ml | 15-25°C/2-8°C |
| RNase A (10 mg/ml) | 0.3 ml | 0.6 ml | –20°C |
| Lysis Blue | 30 ml | 60 ml | 15-25°C |
| Neutral B2 | 30 ml | 60 ml | 15-25°C |
| EQ | 30 ml | 60 ml | 15-25°C |
| Wash PL | 50 ml | 100 ml | 15-25°C |
| EL | 25 ml | 50 ml | 15-25°C |
| Suspension | 5 ml | 10 ml | 15-25°C |
| AnEx Midi Columns | 4 | 8 | 15-25°C |
| Protocol | 1 | 1 | |

## *Equipment and reagents to be supplied by the experimenter*

1. Standard microbiological equipment for growing and harvesting bacteria.
2. Refrigerated centrifuge capable of ≥20,000 x g with a rotor for the appropriate centrifuge tubes or bottles.
3. Isopropanol, ethanol 70%.
4. Disposable gloves, sterile pipet tips, sterile 50 ml tubes.

# Introductory Notes

**NOTE 1 · Kit Specification.** The kit is designed for the isolation of ultrapure plasmid DNA from various species of Gram – bacteria, including recombinant *Escherichia coli* strains, using anion-exchange technology. Obtained plasmid DNA is suitable for use in routine molecular biology applications such as PCR, sequencing, transfection, *in vitro* transcription and translation, and enzymatic modifications.

**NOTE 2 · Maximum Sample Amount.** The maximum column binding capacity for plasmid DNA is 150 µg. Actual yields will depend on culture volume, culture medium, plasmid copy number, size of insert, and host strain. Use either stationary phase or log phase bacterial cultures for obtaining highest DNA quality. Due to differences in growth characteristics of bacteria species, it is recommended to perform a preliminary experiment for determining the optimal starting amount. The protocol is optimized for use with cultures grown in standard Luria Bertani (LB) medium, grown to a cell density of approximately 1–5 x $10^9$ cells per ml. In general, the wet weight of the cell pellet should not exceed 3 g per 1 L culture volume.

**NOTE 3 · Additional Remarks.** Add 100 mg RNase A per liter of CellRes buffer for final concentration of 100 µg/ml.

**NOTE 4 · Kit Compounds Storage.** Once the kit is unpacked, store components at room temperature, except RNaseA, which should be stored at -20°C. After adding RNase A, CellRes buffer should be stored at 2–8°C and is stable for 6 months. In case of occasional buffer ingredients precipitation, simply warm up in 37°C water bath, until clarified.

**NOTE 5 · 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.

Plasmid DNA contaminants such as: RNA, single-stranded DNA, enzymes/proteins, endotoxins (LPS), lipids, dyes, detergents, nucleotides, EDTA, problematic restriction and ligation inhibitors are effectively removed from crude bacterial lysate. The anion exchange resin used for construction of the column is especially optimized toward removal of bacterial endotoxins and problematic inhibitors of restriction of DNA as well as non-specific endo- and exonucleases. Coloured lysis buffer helps in monitoring cell solubilization progress. Optimized low salt buffer provide selective conditions for binding plasmid DNA to the resin. During the gravity flow the contaminants pass through the column and any contaminants remaining on the resin are efficiently removed in wash step. High-quality plasmid DNA is then eluted in high salt buffer. After precipitation isolated DNA is ready for downstream application.

# Protocol

## Part I Preparation and clearing of bacterial lysate

1. Pour cells from overnight culture (11-14 h) into appropriate tube. Harvest by centrifugation at app. 5 000 x g for 10 min at 4°C. Pour off the supernatant and blot tubes upside-down on paper towel to remove any remaining media.

   - *Use up to 25 ml volume culture for high copy plasmids and up to 100 ml volume for low copy plasmids.*

   - *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.*

2. Add 5 ml of **CellRes** buffer and completely resuspend the cell pellet.

   - *Ensure that RNase A is added to CellRes buffer (see page 3, Note 3).*

3. Add 5 ml of blue-coloured **Lysis Blue** buffer. Mix gently, but completely by several-fold inverting, until uniform blue colour of cell resuspension is obtained. Incubate at room temperature for 5 min.

   - *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.*

4. Add 5 ml of neutralization buffer **Neutral B2**. Mix by several-fold inverting, until blue colour will disappear. Incubate at 4°C for 15 min.

5. Centrifuge at app. 20 000 x g for 30 min at 4°C. Remove supernatant containing plasmid DNA very carefully, without disturbing the precipitate.

6. Centrifuge supernatant again at app. 20 000 x g for 15 min at 4°C. Remove supernatant containing plasmid DNA very carefully, without disturbing the precipitate.

   - *This second centrifugation step should be carried out in case of still floating residues to avoid applying suspended material to a column. If the supernatant obtained at point 5 is clear, skip this step.*

## Part II Binding to the resin, washing and elution of plasmid DNA

1. Equilibrate column by applying 5 ml **EQ** buffer and allow the column to empty by gravity flow. Allow the column to drain completely.
2. Apply a clear supernatant from step 6 to the DNA binding column and allow it to enter the resin by gravity flow.
3. Wash column with 10 ml **Wash PL** buffer.
4. Elute DNA with 5 ml **EL** buffer. Collect the eluate in an appropriate tube.
5. Precipitate DNA by adding 3.5 ml (0.7 volumes) of isopropanol to the eluted DNA. Mix and centrifuge immediately at 15 000 x g for 30 min at 4°C.
6. Carefully decant the supernatant. Wash the pellet once with 2 ml of room-temperature 70% ethanol and centrifuge at 15 000 x g for 10 min.
7. Carefully decant the supernatant without disturbing the pellet. Vacuum or air-dry the pellet and dissolve in suitable volume (0.3–1 ml) of **Suspension** buffer (10mM Tris pH 8.5).

# Safety Information

## Lysis Blue / Neutral B

**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.

## EQ / Wash PL / EL

**Warning**

**H226** 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.
**P233** Keep container tightly closed.
**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.
**P303**+**P361**+**P353** If on skin (or hair): take off immediately all contaminated clothing. Rinse skin with water [or shower].

## *AnEx Plasmid Midiprep DNA Purification Kit is designed to isolate ultrapure 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 are effectively removed from crude bacterial lysate. The anion exchange resin used for construction of the column is especially optimized toward removal of problematic inhibitors of restriction of DNA as well as non-specific endo- and exonucleases. Coloured lysis buffer helps in monitoring cell solubilization progress. Optimized low salt buffer provide selective conditions for binding plasmid DNA to the resin. During gravity flow contaminants pass through the column and traces of contaminants remaining on the resin are efficiently removed in wash step. High-quality plasmid DNA is then eluted in high salt buffer. After precipitation isolated DNA is ready for downstream application.

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