DANAGENE PLASMID MIDI/MAXI KIT

REF.0702.4 25 Midis
REF.0702.5 10 Maxis

1. INTRODUCCION

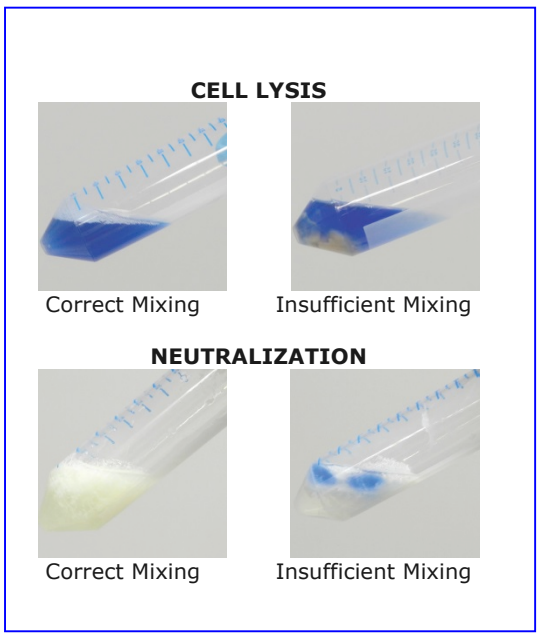
DANAGENE PLASMID Midi/Maxiprep Kit offers a simple method for isolating **plasmid DNA** from **25-500 ml of recombinant E.coli cultures.**

This kit combines a modified alkaline lysis method with the convenience of **anion-exchange columns** to isolate high purity **transfection grade plasmid DNA** from bacterial cell lysates.

During the cell lysis step, both chromosomal and plasmid DNA are denatured. Potassium acetate is added to form a neutralized precipitate containing chromosomal DNA and other cellular components.
Plasmid DNA remains in the solution, reverts to its native supercoiled structure, and is then loaded onto an equilibrated anion-exchange column. The plasmid DNA becomes bound to the anion-exchange resin and is then eluted from the column with washing steps. Eluted DNA is precipitated and easily dissolved in TE buffer or nuclease-free-water.

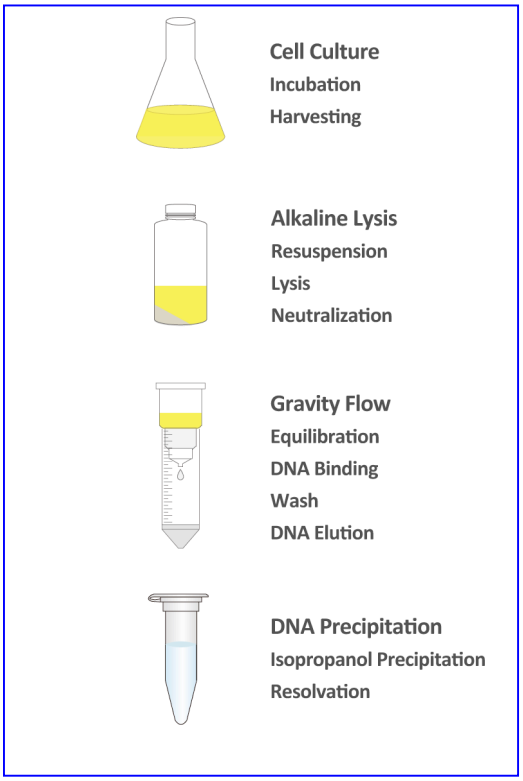
The purified plasmids are suitable for use in the most demanding molecular biology applications, including transfection, in vitro transcription, automated or manual sequencing, cloning, hybridization and PCR
assured of maximum product yield.

Visualization of efficient cell lysis and SDS precipitation using
TrueBLUE Lysis control reagent



Features:

- Based on familiar anion-exchange columns.
- Achieve transfection grade plasmid purity.
- Columns available in MIDI (25/50 ml high-copy plasmids / 100ml low-copy plasmids) and MAXI (100/ 150 ml high-copy plasmids / 500ml low-copy plasmids) formats.
- Each kit includes gravity-flow columns and all the necessary reagents for ultrapure plasmid purification.
- Includes specialized filters to optional remove cellular debris from lysates.



2. KIT COMPONENTS

2.1

	Ref. 0702.4 25 Midipreps	Ref. 0702.5 10 Maxipreps	Ta
Resuspension Buffer	110 ml	110 ml	4ºC
Lysis Buffer	110 ml	110 ml	RT
Neutralization Buffer	110 ml	110 ml	4ºC
Equilibration Buffer	130 ml	130 ml	RT
Wash Buffer	360 ml	360 ml	RT
Elution Buffer	220 ml	130 ml	RT
RNase A (50 mg/ml)	200 µl	200 µl	4ºC
Midi/Maxi Columns	25 unid.	10 unid.	RT
Folded Filters	25 unid.	10 unid.	RT
TRUEBLUE Lysis control reagent	1500 µl	1500 µl	RT

Equipment and aditional reagents required

* 70 % Ethanol
* Centrifuge and tubes for harvesting bacterial cultures, and precipitate DNA, capable to spin speed of >15.000 xg
* Tubes for collecting and precipitating eluted plasmid DNA
* 100 % Isopropanol
* TE Buffer

3. PROTOCOL

3.1 Preliminary Conditions

Several factors can interfere in the plasmid DNA obtaining. These include the number of vector copies, the DNA insert, the host cell, growing conditions and medium.

	MIDI	MAXI
Binding Capacity	100 μg	500 μg
High-copy plasmids culture volume	25-50 ml	100-150 ml
Low-copy plasmids culture volume	100 ml	500 ml

3.2 Preliminary Preparations

- Add provided RNase A to the Resuspension Solution and store at 4ºC.
- Verify that the Lysis Solution does not contain precipitated SDS due to the low temperatures. If necessary, dissolve the SDS heating at 37°C.

3.3 Protocol for plasmid DNA extraction from HIGH-COPY PLASMID (> 20 copies/cell).

MIDI (25-50ml) / MAXI 100-150 ml)

1. **Prepare an overnight culture:** Begin with an isolated bacterial colony from a fresh plate and inoculate a starter culture of 2-5 ml LB medium containing the appropriate antibiotic(s). Incubate for approx. 8 h at 37ºC with vigorous shaking (approx.300 rpm). Dilute the starter culture 1/500 to 1/1000 into selective medium, grow at 37ºC for 12-16 h with vigorous shaking (approx.300 rpm).

2. **Harvest bacterial cells** from an LB culture by centrifugation at **6,000 x g for 15 min at 4°C.** Carefully discard the supernatant.

3. **Cell Lysis:**
   Resuspend the pellet of bacterial cells in **4 ml / 10 ml** of **Resuspension Buffer (+ RNase A) + 40 μl/ 100 μl of TRUELBLUE Lysis control reagent by vigorous vortexing, assure the complete cells resuspension.**

NOTE: Make sufficient Resuspension buffer /TRUEBLUE Lysis control reagent working solution for the number of plasmid preps being performed. TRUEBLUE precipitates after addition, this precipitate will completely dissolve after addition Lysis Buffer. Shake before use to resuspend the TRUEBLUE particles.

4. Add **4 ml / 10 ml** of **Lysis Buffer** to the suspension. Mix gently by inverting the tube 6-8 times. Incubate the mixture at room temperature (20-25°C) for 3 min (max 5 min). Do not vortex since this will release contaminating chromosomal DNA from the cellular debris into the suspension.

5. Add **4 ml / 10 ml** of **pre-cooled Neutralization Buffer** (4°C) to the suspension . Immediately mix the lysate by gently inverting the tube 6-8 times until a homogeneous suspension containing an off-white flocculate is formed. **Incubate the suspension on ice for 10-15 min.** Equilibrate the column during this time.

6. **Equilibrate the column:** Equilibrate a **MIDI / MAXI** Column with **5 ml / 10 ml** of **Equilibration Buffer**. Allow the column to empty by gravity flow. Discard the flow-through.

7. **Clarify the lysate:** Clear the bacterial lysate by following either **Option 1** or **Option 2** described below. **This step is extremely important;** excess flocculate left in the suspension may clog the Column in later steps.
   7.1. **Option 1: Filter the suspension.** Place Folded Filter in a small funnel for support and pre-wet the filter with a few drops of Equilibration Buffer or nuclease-free H2O. Load the bacterial lysate onto the pre-wet filter and collect the flow-through into a clean, nuclease-free tube. This method produces a clean lysate but the yield of plasmid DNA can be e smaller than with the centrifugation method.
   7.2. **Option 2: Centrifuge the suspension.** Centrifuge at >15,000 x g for 20 **min / 30 min at** 4°C. If the suspension contains residual flocculate after the first centrifugation, repeat this step.

8. **Bind plasmid to column:** Load the cleared lysate from Step 7 onto the equilibrated Column. Allow the column to empty by gravity flow.

9. **Wash the column** with **12 ml / 36 ml** of **Wash Buffer** Discard flow-through.

10. **Elute the plasmid DNA** with **8 ml / 12 ml** of **Elution Buffer** and collect the sample by gravity flow into a clean, nuclease-free tube. Precipitate the elute as soon as possible, however, it may be stored in a closed tube at 4°C for several hours. In this case, it is very important to **pre-warm the elute to room temperature** before the plasmid DNA is precipitated.

11. **Precipitate DNA:** Add **6 ml / 9 ml of room-temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at >3000 rpm x g for 20 minutes **(preferably at 15,000 x g for 20 min) at 4°C.** Carefully discard the supernatant.

12. **Wash and dry DNA pellet:** Add **2 ml / 5 ml of room-temperature 70% ethanol** to the pellet as indicated below. Vortex briefly and at >3000 rpm x g for 10 minutes **(preferably at 15,000 x g for 10 min).** Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to air dry **10-20 min at room temperature (20-25°C)**, no less than the indicated time. Do not over-dry the pellet as the DNA will become difficult to resuspend.

13. **Resuspend DNA** in an appropriate volume of TE Buffer or nuclease free H2O with constant, gentle shaking for 10-60 min or redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tube has been used.

3.4 Protocol for plasmid DNA extraction from LOW-COPY PLASMID (< 20 copies/cell).

MIDI (10-100 ml) / MAXI (100-500 ml)

If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes in order to increase the efficiency of alkaline lysis, and thereby the DNA yield. In case additional Buffers are needed, their compositions are provided in Appendix A

1. **Prepare an overnight culture:** Begin with an isolated bacterial colony from a fresh plate and inoculate a starter culture of 2-5 ml LB medium containing the appropriate antibiotic(s). Incubate for approx. 8 h at 37ºC with vigorous shaking (approx.300 rpm). Dilute the starter culture 1/500 to 1/1000 into selective medium, grow at 37ºC for 12-16 h with vigorous shaking (approx.300 rpm).

2. **Harvest bacterial cells** from an LB culture by centrifugation at **6,000 x g for 15 min at 4°C.** Carefully discard the supernatant.

3. **Cell Lysis:**

Resuspend the pellet of bacterial cells in **8 ml / 24 ml** of **Resuspension Buffer (+ RNase A) + 80 µl/ 240µl of TRUEBLUE Lysis control reagent by vigorous vortexing**, **assure the complete cells resuspension.**

NOTE: Make sufficient Resuspension Buffer/ TRUELUE Lysis control reagent working solution for the number of plasmid preps being performed. TRUEBLUE precipitates after addition, this precipitate will completely dissolve after addition Lysis Buffer . Shake before use to resuspend the TRUEBLUE particles.

4. Add **8 ml / 24 ml** of **Lysis Buffer** to the suspension. Mix gently by inverting the tube 6-8 times. Incubate the mixture at room temperature (20-25°C) for 2–3 min (max 5 min). Do not vortex since this will release contaminating chromosomal DNA from the cellular debris into the suspension.

5. Add **8 ml / 24 ml** of **pre-cooled Neutralization Buffer** (4°C) to the suspension . Immediately mix the lysate by gently inverting the tube 6-8 times until a homogeneous suspension containing an off-white flocculate is formed .**Incubate the suspension on ice for 5 min.** Equilibrate the column during this time.

6. **Equilibrate the column:** Equilibrate a **MIDI / MAXI** Column with **5 ml / 10 ml** of **Equilibration Buffer (PEQ)** Allow the column to empty by gravity flow. Discard the flow-through.

7. **Clarify the lysate:** Clear the bacterial lysate by following either **Option 1** or **Option 2** described below. **This step is extremely important;** excess flocculate left in the suspension may clog the Column in later steps.
   7.1. **Option 1: Filter the suspension.** Place Folded Filter in a small funnel for support and pre-wet the filter with a few drops of Equilibration Buffer or nuclease-free H2O. Load the bacterial lysate onto the pre-wet filter and collect the flow-through into a clean, nuclease-free tube. This method produce a clean lysate but the yield of plasmid DNA can be e smaller than with the centrifugation method.
   7.2. **Option 2: Centrifuge the suspension.** Centrifuge at >15,000 x g for 20 **min / 30 min at** 4°C. If the suspension contains residual flocculate after the first centrifugation, repeat this step.

8. **Bind plasmid to column:** Load the cleared lysate from Step 7 onto the equilibrated Column. Allow the column to empty by gravity flow.

9. **Wash the column** with **12 ml / 30 ml** of **Wash Buffer** . Discard flow-through.

10. **Elute DNA** with **8 ml / 12 ml** of **Elution Buffer** and collect the sample by gravity flow into a clean, nuclease-free tube. Precipitate the elute as soon as possible, however, it may be stored in a closed tube at 4°C for several hours. In this case, it is very important to **pre-warm the elute to room temperature** before the plasmid DNA is precipitated.

11. **Precipitate DNA:** add 6 **ml / 9 ml** of room**-temperature isopropanol** to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at >3000 rpm x g for 20 minutes **(preferably at 15,000 x g for 20 min) at 4°C.** Carefully discard the supernatant.

12. **Wash and dry DNA pellet:** add **2 ml / 5 ml** of room temperature **70% ethanol** to the pellet. Vortex briefly and at >3000 rpm x g for 10 minutes **(preferably at 15,000 x g for 10 min).** Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to air dry **10-20 min at room temperature (20-25°C)**, no less than the indicated time. Do not over-dry the pellet as the DNA will become difficult to resuspend.

13. **Resuspend DNA:** Redissolve the DNA pellet in an appropriate volume of TE Buffer or nuclease free H2O with constant, gentle shaking for 10-60 min or redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tube have been used.

4. APPENDIX

Resuspension Buffer: 50mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 μg Rnase A.
Dissolve 6.06 gr Tris base, 3.72 gr $Na_2EDTA.2H_20$ in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 litre with distilled water. Add 100 mg Rnase A.
Lysis Buffer: 200 mM NaoH, 1% SDS (w/v)
Dissolve 8.0 gr NaOH pellets in 950 ml distilled water and add 50 ml of a 20% SDS (w/v) solution. The final volume should be 1 litre.
Neutralization Buffer: 3.0 M potassium acetate, pH 5.5
Dissolve 294.5 gr potassium acetate in 500 ml distilled water. Adjust the pH to 5.5 with glacial acetic acid (approx 110 ml). Adjust the volume to 1 litre with distilled water.

5. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es