Data sheet

WideUSE plasmid Midi/Maxiprep kit

Cat. No: AN0168 (MidiPrep: 25 reactions)
Cat. No: AN0169 (MaxiPrep: 10 reactions)

Description

WideUSE plasmid Midi/Maxiprep kit offers a simple and convenient method for the routinary isolation of high quality plasmid preparations in Midiprep/Maxiprep format.

It introduces the Lysis control reagent (LCR) a color indicator which provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA and cell debris. This makes ideal for use by researchers who have not much experience with plasmid preparation as well as experienced scientists who want to be assured of maximum product yield.

Features

- No phenol-chloroform extraction.
- **Ready to use** plasmid DNA.
- **Just a few minutes** procedure.
- **Midi and maxi format.**
- Based on familiar **anion-exchange columns**.
- Achieve transfection grade plasmid purity.
- Includes specialized filters to optional remove cellular debris from lysates.

Kit Components

Item	AN0168	AN0169
Resuspension Solution	110 ml	110 ml
Lysis Solution (BLY)	110 ml	110 ml
Neutralization Solution	110 ml	110 ml
Equilibration Buffer	130 ml	130 ml
Wash Buffer (WB1)	360 ml	360 ml
Elution Buffer (EB)	220 ml	130 ml
Lysis control reagent (LCR)	1500 µl	1500 µl
TE Buffer	25 ml	25 ml
Plasmid Midi / Maxi Columns	25	10
RNase (50 mg/ml)	200 µl	200 µl
Folder Filters	25	10

Storage

WideUSE plasmid Midi/Maxiprep kit should be stored at room temperature (15–25°C) for up to 12 months without any reduction in performance. Store the RNAse A at -20ºC. After addition of RNase A to Resuspension Solution can be stored at 4ºC.

Verify that the Lysis Solution (BLY) does not contain precipitated SDS due to the low temperatures. If necessary, dissolve the SDS heating at 37°C.

Quality Certifications

WideUSE Plasmid Purification Kit is tested for the isolation of any plasmid DNA from transformed *E.coli*.

The quality of purified DNA is analysed by:

- Ratio 260/ 280.
- Agarose gel electrophoresis.
- Digestion with restriction endonucleases

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Protocol: Plasmid DNA Purification

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.

	MIDIPREP	MAXIPREP
Binding Capacity	100 µg	500 µg
High-copy plasmids culture volume	25 ml	100 ml
Low-copy plasmids culture volume	100 ml	500 ml

PREPARATION OF WORKING SOLUTIONS

Before starting the protocol prepare the following reagent:

✓ Add provided RNase A to the Resuspension Solution and store at 4ºC.

ASSAY PROCEDURE

A. Protocol for plasmid DNA 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 Solution (+ RNase A) + 40 µl/ 100 µl of Lysis control reagent by vigorous vortexing, assure the complete cells resuspension.**
   NOTE: *Make sufficient Resuspension solution / Lysis control reagent working solution for the number of plasmid preps being performed. LCR precipitates after addition to Resuspension solution, this precipitate will completely dissolve after addition BLY. Shake before use to resuspend the LCR particles.*

4. Add **4 ml / 10 ml** of **Lysis Solution (BLY)** 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 H_2O. 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 smaller than with the centrifugation method.

7.2. **Option 2: Centrifuge the suspension.** Centrifuge at >15,000 x g for **30 min / 40 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 (WB1).** Discard flow-through.

10. **Elute the plasmid DNA** with **8 ml / 12 ml** of **Elution Buffer (EB)** 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 **15,000 x g for 30 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 centrifuge 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 H_2O 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.

B. Protocol for plasmid DNA from LOW-COPY PLASMID < 20 copies/cell

MIDI (10-100ml) / 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.

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 solution (+ RNase A) (PM1) + 80 µl/ 240 µl of Lysis control reagent by vigorous vortexing, assure the complete cells resuspension.**
   NOTE: *Make sufficient Resuspension solution / Lysis control reagent working solution for the number of plasmid preps being performed. LCR precipitates after addition to Resuspension solution, this precipitate will completely dissolve after addition BLY. Shake before use to resuspend the LCR particles.*

4. Add **8 ml / 24 ml** of Lysis Buffer (BLY) 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 **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 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 H_2O. 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 smaller than with the centrifugation method.

   7.2. **Option 2: Centrifuge the suspension.** Centrifuge at >15,000 x g for **30 min** / **40 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 (WB1). Discard flow-through.

10. **Elute the plasmid** DNA with **8 ml** / **12 ml** of Elution Buffer (EB) 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 15,000 x g for 30 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 centrifuge 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 H_2O 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.

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