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Product Information

GenElute™ Plasmid Midiprep Kit

Catalog Number **PLD35**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

The GenElute™ Plasmid Midiprep Kit offers a simple, rapid, cost-effective method for isolating plasmid DNA from recombinant *E. coli* cultures. By combining silica-binding technology and the convenience of a spin column format, up to 300 µg of plasmid DNA can be recovered from 20–40 ml of Luria Broth (LB) in about 45 minutes. Note that actual yield and optimal volume of culture to use depend on the plasmid and the culture medium (see Procedure, step 1).

An overnight recombinant *E. coli* culture is harvested by centrifugation and subjected to a modified alkaline-SDS lysis procedure followed by adsorption of the DNA onto silica in the presence of high salts.[1,2] Contaminants are then removed by a spin-wash step. Finally, the bound DNA is eluted in water or Tris-EDTA buffer.

The recovered plasmid DNA is predominately in its supercoiled form. There is no visual evidence of genomic DNA or RNA contamination detected by agarose gel electrophoresis. The DNA is ready for immediate use in downstream applications such as restriction digestion, ligation, sequencing, PCR, and transfection.

Components

Reagents Provided	Catalog Number	PLD35 35 Reactions
Resuspension Solution	R1149	100 ml
RNase A Solution	R6148	0.6 ml
Lysis Solution	L1912	100 ml
Neutralization/Binding Solution	N5158	65 ml
Column Preparation Solution	C2112	225 ml
Optional Wash Solution	W4011	80 ml
Wash Solution Concentrate	W3886	25 ml
Elution Solution (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)	E5650	45 ml
GenElute Midiprep Binding Columns in Tubes	G6540	35
Collection Tubes, 15 ml capacity	C4228	2 × 35

Reagents and Equipment Required but Not Provided.

- Ethanol (95–100%, Catalog Numbers E7148, E7023, or 459836)
- Centrifuge with swinging bucket rotor capable of 3,000–5,000 × *g*
- Centrifuge capable of 12,000–15,000 × *g*
- Centrifuge tubes, Oak Ridge style, Catalog Number T2918

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. **Thoroughly mix reagents** – Examine reagents for precipitation. If any kit reagent forms a precipitate upon storage, warm at 55–65 °C until the precipitate dissolves. Allow the reagent to cool to room temperature before use.

2. **Resuspension Solution** – Spin the tube of RNase A solution briefly. Add 500 µl of the RNase A Solution to the Resuspension Solution prior to initial use.

3. **Wash Solution** – Dilute the Wash Solution Concentrate with 100 ml of 95–100% ethanol prior to initial use. After each use, tightly cap the diluted wash solution to prevent the evaporation of ethanol.

Storage/Stability

Store the kit at room temperature.

If any kit reagent forms a precipitate upon storage, warm at 55–65 °C until the precipitate dissolves. Allow the reagent to cool to room temperature before use.

Procedure

Note: All centrifugation speeds are given in units of *g*. Please refer to Table 1 for information on converting *g*-force to rpm. If centrifuges/rotors for the required *g*-forces are not available, use the maximum *g*-force possible and increase the spin time proportionally. Spin until all liquid passes through the column. A swinging bucket rotor is necessary for steps 5–9.

Table 1.
Conversion of Centrifugal Force (in units of *g*) to rpm for Common Rotors

Centrifuge	Rotor	Type*	Radius (cm)	rpm at 3,000 × *g*	rpm at 5,000 × *g*	rpm at 15,000 × *g*
Beckman						
Allegra 6	GH-3.8	SB	20.4	3,631	4,688	N/A
Allegra 21(R)	S4180	SB	16.1	4,081	5,268	N/A
Allegra 64	F0485	FA	9.0	**N/A	N/A	12,211
	F0685	FA	9.7	N/A	N/A	11,764
TJ-25	TS-5.1-500	SB	19.0	3,756	4,849	N/A
	TA-10-250	FA	13.7	N/A	N/A	9,901
Rotors for older Beckman centrifuges	JA-10	FA	15.8	N/A	N/A	9,215
	JA-14	FA	13.7	N/A	N/A	9,896
	JA-20	FA	10.8	N/A	N/A	11,146
	JS-13	FA	14.0	N/A	N/A	9,790
IEC						
MP4(R)	215	SB	13.0	4,537	5,857	N/A
	224	SB	35.9	2,733	3,528	N/A
PR-7000M	966	SB	24.5	3,310	4,274	N/A
B22M	877	FA	12.6	N/A	N/A	10,318
Sorvall						
	HB-4	SB	14.7	4,277	5,522	N/A
	HB-6	SB	14.6	4,284	5,531	N/A
	HS-4	SB	17.2	3.948	5,097	N/A
	SH-80	SB	10.1	5,142	6,639	N/A
	GSA	FA	14.5	N/A	N/A	9,604
	SA-300	FA	9.7	N/A	N/A	11,784
	SA-600	FA	12.9	N/A	N/A	10,179
	SE-12	FA	9.3	N/A	N/A	11,997
	SL-50T	FA	10.7	N/A	N/A	11,203
	SS-34	FA	10.7	N/A	N/A	11,203

* SB = swinging bucket; FA = fixed angle;
** N/A = not appropriate for application

See the previous table for spin speeds in rpm for selected common centrifuges and rotors. The correct rpm for unlisted rotors can be calculated using the formula:

$$RPM = \sqrt{RCF / 1.118 \times 10^{-5} r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force) in units of *g*; *r* = radius of the rotor in cm; *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

All steps are carried out at room temperature.

1. **Harvest cells** – Pellet 5–40 ml of overnight recombinant *E. coli* culture by centrifugation. Optimal volume of culture to use depends upon the culture density. For best yields, follow the instructions in the note below. Transfer the appropriate volume of the recombinant *E. coli* culture to an Oak Ridge style tube (capable of ≥15,000 × *g*) and centrifuge at 3,000–5,000 × *g* for 5–10 minutes. Remove and discard all of the medium supernatant.

   Note: For best results, start with a single colony from a freshly streaked plate. Grow in Luria broth (LB) containing the appropriate antibiotic at 37 °C with vigorous shaking (250–300 rpm) overnight. Measure the absorbance of the overnight culture at 600 nm. Use a total cell mass of ~80, where cell mass equals OD_{600} × ml of culture. To calculate the volume of culture to use, take the desired cell mass (80) and divide by the absorbance of the overnight culture at 600 nm. For example, with a very dense culture of recombinant *E. coli* grown to an OD_{600} of ~4.0, use only 20 ml of the culture. With a less dense culture, where OD_{600} is ~2.0, use 40 ml. For low copy plasmids, use twice as much culture (total cell mass of 160). Do not exceed a total cell mass of 100 (with high copy plasmids) or 200 (with low copy plasmids). Higher cell mass can cause a reduction in yield. For cultures grown in rich media, less volume may be necessary. Please contact Sigma Technical Service if you require further assistance.

2. **Resuspend cells** – Prior to first time use, be sure to add the appropriate volume of the RNase A Solution to the Resuspension Solution. Completely resuspend the bacterial pellet with 1.2 ml of Resuspension Solution by pipetting up and down. Make sure the cells are completely resuspended until homogenous. Incomplete resuspension will result in poor recovery.

3. **Lyse cells** – Lyse the resuspended cells by adding 1.2 ml of the Lysis Solution. Immediately mix the contents by gentle inversion (6–8 times) until the mixture becomes clear and viscous. **Do not vortex**. Harsh mixing will shear genomic DNA, resulting in chromosomal DNA contamination in the final recovered plasmid DNA. **Do not allow lysis to exceed 5 minutes**. Prolonged alkaline lysis may permanently denature supercoiled plasmid DNA, which may render it unsuitable for most downstream applications.

4. **Neutralize** – Precipitate the cell debris by adding 1.6 ml of the Neutralization/Binding Solution.Gently invert the tube 4–6 times. Pellet the cell debris by centrifuging at ≥15,000 × *g* for 10–15 minutes. Cell debris, proteins, lipids, SDS, and chromosomal DNA should fall out of solution as a cloudy, viscous precipitate. If the supernatant contains a large amount of floating particulates after centrifugation, re-centrifuge the supernatant before proceeding to step 5.

5. **DNA Binding Column Preparation** – Insert a GenElute Midiprep DNA Binding Column into a 15 ml collection tube if not already assembled. Add 3 ml of Column Preparation Solution to each column and centrifuge in a swinging bucket rotor at 3,000–5,000 × *g* for 1–2 minutes. Discard the flow-through liquid.

   Note: The Column Preparation Solution maximizes binding of DNA to the filter resulting in more consistent yields. This column preparation step can be conveniently carried out during or prior to step 4.

6. **Load cleared lysate** – Transfer the cleared lysate from step 4 to the prepared column seated in a 15 ml collection tube and centrifuge at 3,000–5,000 × *g* for 1–2 minutes in a swinging bucket rotor. Discard the flow-through liquid.

7. **Optional Wash (use only for $EndA^+$ strains)** – Add 2.0 ml of the Optional Wash Solution to the column. Centrifuge in a swinging bucket rotor at 3,000–5,000 × *g* for 2 minutes. Discard the flow-through liquid.

   Note: When working with bacterial strains containing the wild-type $EndA^+$ gene, such as HB101, JM101, and the NM and PR series, the Optional Wash step is necessary to avoid nuclease contamination of the final plasmid DNA.

8. **Wash column** – Prior to first time use, be sure to add ethanol to the Wash Solution Concentrate. Add 3 ml of the diluted Wash Solution to the column. Centrifuge in a swinging bucket rotor at 3,000–5,000 × *g* for 5 minutes. The column wash step removes residual salt and other contaminants introduced during the column load. Make sure the Wash Solution is completely removed from the column before proceeding to step 9.

9. **Elute DNA** – Transfer the column to a fresh 15 ml collection tube. Add 1 ml of Elution Solution or molecular biology reagent water to the column. For DNA sequencing and other enzymatic applications, use water or 5 mM Tris-HCl, pH 8.0, as an eluant. Centrifuge in a swinging bucket rotor at 3,000–5,000 × *g* for 3–5 minutes. The DNA is now present in the eluate and is ready for immediate use or storage at –20 °C.

   Note: Less than 0.8 ml of eluate may be recovered after centrifugation, particularly if the maximum amount of starting culture was used. However, plasmid DNA yield will not be compromised. For a more concentrated DNA preparation, the elution volume may be reduced to a minimum of 500 µl. For optimal recovery in 500 µl, preheat the elution solution to 65 °C and add directly to the binding filter. Allow the preheated elution solution to soak into the binding filter for 10 minutes before centrifugation. Incubating with preheated elution solution will improve recovery, but the total plasmid DNA yield is likely to be less than with elution in the full 1 ml.

Results

Recovery and purity of the plasmid DNA may be determined by spectrophotometric analysis. The ratio of absorbance at 260 to 280 nm (A_{260}/A_{280}) should be 1.7 to 1.9. Size and quality of DNA may be determined by agarose gel electrophoresis or pulse field electrophoresis.

References

1. Birnboim, H.C., and Doly, J., A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res., **7**, 1513-1522 (1979).
2. Vogelstein, B., and Gillespie, D., Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA, **76**, 615-619 (1979).

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Troubleshooting Guide

Problem	Cause	Solution
Poor or low plasmid DNA recovery	Binding columns were spun in a fixed angle rotor, or with insufficient *g*-force.	Binding columns must be spun in a swinging bucket rotor at 3,000–5,000 × *g* in steps 5–9 for liquids to pass through efficiently. Actual spin speed in rpm will depend on rotor size (see note at beginning of the Procedure section).
	Wash Solution is too concentrated.	Confirm that the Wash Solution concentrate was diluted with the specified volume of ethanol. Keep the bottle tightly capped between uses to prevent evaporation.
	Culture is too old.	Streak a fresh plate from a freezer stock, pick a single colony, and prepare a new culture.
	Too many or too few cells were used.	Confirm cell density by measuring OD_{600}. To calculate the volume of culture to use, take the desired cell mass (80 for high copy or 160 for low copy plasmids) and divide by the absorbance of the overnight culture at 600 nm.
	Plasmid replication is poor.	Confirm that the cells were grown in appropriate medium under optimized conditions.
	Antibiotic activity is insufficient.	Use fresh antibiotic solution for growth of overnight cultures. Most antibiotic solutions are light sensitive and degrade during long term storage at 2–8 °C.
	Alkaline lysis is prolonged.	Reduce the lysis time (step 3) to 3 minutes or until the suspended cells form a clear, viscous solution.
	Precipitation of cell debris is incomplete.	Reduce the initial volume of cell culture.
	Lysis is incomplete.	Reduce the initial volume of culture or increase the lysis time (step 3) while monitoring the lysis visually. Do not exceed a cell mass of 100 (cell mass = OD_{600} × ml of culture). For best results, use a total cell mass of 80 for high copy or 160 for low copy plasmids.
Absorbance of purified DNA does not accurately reflect quantity of plasmid (A_{260}/A_{280} ratio is high or low).	Wash Solution is diluted with ethanol containing impurities.	Check the absorbance of ethanol between 250 and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing. Impurities may show up in the eluate and may contribute to the absorbance in the final product.
	Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient.	Confirm that the RNase A Solution was added to the Resuspension Solution prior to first use. The RNase A Solution may degrade due to high temperatures (>65 °C) or prolonged storage (>6 months at room temperature).
	Plasmid DNA is contaminated with chromosomal DNA.	Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction (step 3) or neutralization procedure (step 4).
	Background reading is high due to silica fines.	Spin DNA sample at maximum speed for 1 minute; use supernatant to repeat absorbance readings.
	Purification is incomplete due to column overloading.	Reduce the initial volume of culture. Do not exceed 100 OD_{600} units of total cell mass (cell mass = OD_{600} × ml of culture). For best results, use a cell mass of 80.

Troubleshooting Guide (continued)

Problem	Cause	Solution
Additional band is migrating ahead of supercoiled plasmid during electrophoresis.	A portion of the plasmid DNA is permanently denatured.	Do not allow the lysis reaction (step 3) to exceed 5 minutes. Note the (covalently open) double-stranded plasmid DNA runs slower than the supercoiled DNA during electrophoresis.
Poor perform-ance in downstream enzymatic applications	Purification is incomplete.	Salts in one or more of the solutions may have precipitated. Heat the solution at 65 °C until dissolved. Cool to room temperature prior to use.
	DNA concentration is too low.	Precipitate the DNA with ethanol, then resuspend the DNA in a smaller volume of water or Elution Solution. **Or** Elute silica-bound DNA with less Elution Solution. Note that using less Elution Solution may reduce the overall recovery.
	DNA was prepared from $EndA^+$ strains.	The Optional Wash (step 7) must be included when recovering DNA from $EndA^+$ strains.
	The final plasmid DNA eluate contains too much salt.	Precipitate the DNA using ethanol. Dry the pellet. Take up in water or Elution Solution. Note the Elution Solution contains EDTA, which may chelate divalent cations (*e.g.*, Mg^{2+}), which are important co-factors for many enzymes.
	The column contains residual ethanol from the diluted Wash Solution.	Re-centrifuge the column for 1 minute after washing (step 8) to remove any residual Wash Solution.