AuPreP™ Plasmid Midi Kit

Cat. #: PMD12-143LT (25 preps/Kit)
PMD12-145LT (50 preps/Kit)

AuPreP™ Plasmid Midi Kit is designed for rapid isolation of plasmid DNA of superior quality from an average of 25-100 ml bacterial culture. With AuPreP™'s proprietary anion-exchange resin, up to 100 µg of plasmid DNA ideal for cell transfection can be isolated from bacterial cells in less than 2 hours without use of toxic chemicals such as phenol, chloroform, CsCl, and ethidium bromide .

Plasmid Midi Kit

Culture (high copy plasmid)	25-50 ml
(low copy plasmid)	50-100 ml
Binding capacity	up to 100 µg
Elution volume	100 µl
Preparation Time	

Downstream Applications

* Transfection
* Radioactive and Fluorescent sequencing
* Restrictive enzymatic digestion
* Transformation
* Ligation
* Microinjection
* PCR

Kit Contents:

	PMD12-143LT** (25 preps)	PMD12-145LT** (50 preps)
VP1 Buffer*	120 ml	240 ml
VP2 Buffer	120 ml	240 ml
VP3 Buffer	120 ml	240 ml
VP4 Buffer	265 ml	265 ml x 2
VP5 Buffer	225 ml x 2	265 ml
VP6 Buffer	130 ml	265 ml
RNase A, 100 mg/ml	120 µl	240 µl
Plasmid Midi Column	25 pieces	50 pieces
Filter Column	30 pieces	50 pieces
Protocol	1	1

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*Add RNase A into VP1 Buffer before use and store at 4°C (refer to **Important Notes**, No. 3, page 7). The final concentration of RNase A in VP1 Buffer is 100 µg/ml.

Volumes of buffers provided in PMD12-143LT and PMD12-145LT are enough for regular preparation of **high copy plasmids from a **25-50 ml** culture. Where there is a requirement of additional buffers for preparation of low copy plasmids from a larger volume of culture or for other applications, separate order for extra buffers and RNase A will be required according to user's need.

Buffers and RNase A are available for separate purchase.

Shipping and Storage

All components of AuPreP™ Plasmid Midi Kit are stable at 20-25°C for one year. If room temperature is always above 25°C, RNase A solution is better be stored at 4°C.

Notes:

Please read the following notes before starting the procedures.

1. Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.

2. Briefly centrifuge RNase A tube to bring down the solution. Add 1 ml of VP1 Buffer into RNase A solution and mix well. Transfer the mixture into VP1 Buffer bottle and store at 4°C.

3. If precipitation forms in VP2 Buffer, incubate at 55°C for 10 minutes to redissolve the salt precipitates. Do **not** shake VP2 Buffer, SDS present will lead to serious foaming.

4. Pre-chill VP3 Buffer on ice or at 4°C before use.

5. Prepare a 4°C centrifuge before starting the procedure.

6. Prepare room-temperature isopropanol and 70% ethanol before starting the procedure.

Protocol:

1. Inoculate 1-3 ml LB medium containing appropriate antibiotic(s) with plasmid-containing bacteria from a single colony on a fresh plate or a glycerol stock. Incubate this starter culture at 37°C for 8-16 hours with vigorous agitation.	

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2. Dilute the starter culture by 500 folds in 25-50 ml (for high copy plasmid) or 50-100 ml (for low copy plasmid) LB medium containing appropriate antibiotic(s). Incubate at 37°C overnight (12-16 hours) with vigorous agitation.	If the bacterial cells are grown more than 16 hours, over-grown cells usually have reduced yield of plasmids. If use more than 50 ml culture for plasmid extraction, add **double** volumes of **VP1, VP2,** and **VP3 Buffer** to ensure complete cell lysis. To increase yield of low copy plasmid, refer to **AuPreP™'s Hints**, No. 2, page 16.
3. Harvest the cells by centrifuging at 6,000 x g for 15 minutes. Decant the supernatant and remove all medium residue by pipet.	Make sure that cells are well-pelleted at the bottom of the centrifuge bottle.
4. Add 4 ml of VP1 Buffer to the pellet, and resuspend the cells completely by vortexing or pipetting.	Make sure that RNase A has been added into VP1 Buffer when first open (refer to **Important Notes**, No. 2, page 7). **No** cell clump should be visible after resuspension of the cells. Clumped cells cannot be lysed well.
5. Add 4 ml of VP2 Buffer and **gently** mix (invert and rotate the tube or bottle) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 5 minutes.	Do **not** vortex! Vortexing shears genomic DNA and leads to chromosomal DNA contamination. If necessary, inverting and rotating the tube until the lysate becomes clear and viscous. Do **not** incubate in VP2 Buffer for more than 5 minutes.
6. Add 4 ml ice-cold VP3 Buffer, mix the solution immediately and gently. A white precipiate should be formed.	Pre-chill VP3 Buffer before use. Addition of VP3 without immediate mixing will result in uneven precipitation.
7. (Optional) Incubate on ice for 20 minutes.	Incubation on ice is optional. This step facilitates more complete precipitation of cell debris, proteins, chromosomal DNA, and detergent for subsequent removal by centrifugation.
8. Equilibrate a Midi-V100 Column by applying 10 ml of VP4 Buffer and allow the buffer to flow through the column by gravity. Discard the flow-through.	

9. Centrifuge the precipitate lysate from Step 6 or 7 at 20,000 x g for 30 minutes at 4°C.	20,000 x g is equivalent to 12,000 rpm in Beckman JA-17 rotor and 13,000 rpm in Sorvall SS-34 rotor. A compact white pellet should be formed after centrifugation.
10. After centrifugation, immediately load the supernatant into the column. Allow it to flow through by gravity. Discard the flow-through.	Be careful not to transfer any white pellet into the column to avoid clogging of the column. If some pellet accidentally gets into the column, use a clean sterile pipette tip to take as much out as possible.
11. Wash the column by adding 15 ml of VP5 Buffer into the column and allow it to flow through by gravity. Discard the flow-through.	
12. Add 5 ml VP6 Buffer into the column to elute plasmid DNA by gravity flow.	
13. Precipitate plasmid DNA by adding 3.75 ml (0.75 volume) of room-temperature isopropanol to the DNA eluant. Mix and centrifuge at μ 15,000 x g for 30 minutes at 4°C. Remove the supernatant carefully.	15,000 x g is equivalent to 9,500 rpm in Beckman JA-17 rotor and 11,000 rpm in Sorvall SS-34 rotor. A transparent layer of plasmid DNA pellet formed after centrifugation can be hardly visible. Be careful NOT to pour it off together with the supernatant.
14. Wash the DNA pellet with 5 ml of room-temperature 70% ethanol and centrifuge at μ15,000 x g for 10 minutes. Remove the supernatant carefully.	Room-temperature 70% ethanol removes salts more completely than ice-cold 70% ethanol.
15. Allow the DNA pellet to air dry for 10 minutes. Redissolve the DNA in 100 μl or a suitable volume of ddH2O, TE buffer, or 10 mM Tris-HCl (pH 7-8.5).	For long-term storage, TE buffer should be used for elution. However, since EDTA in TE buffer may affect further enzymatic reaction, ddH2O or 10 mM Tris-HC is preferred.
16. Load the DNA solution into a Filter Column sitting in a 2-ml sterile eppendorf tube. Centrifuge the column at full speed in a microcentrifuge for 20 seconds. Collect the flow-through plasmid solution.	This step removes any resin residue possibly eluted out with the plasmid.
17. Store plasmid DNA at 4°C or -20°C.	

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Troubleshooting:

Problem	Possible Reason	Solution
Poor bacterial growth	Inoculate bacterial cells directly from a plate or glycerol stock stored for a long time	Always inoculate a big volume of LB medium with a fresh starter culture as indicated in the protocol.
	Incubation with inadequate shaking	Grow cells with vigorous shaking (e.g. 250-300 rpm). Adjust a suitable shaking speed according to the angular magnitude of the shaker platform.
Poor cell lysis	Use too many bacterial cells harvested from a big volume of culture or an over-grown culture	Up to 50 ml culture for high copy plasmid. Up to 100 ml culture for low copy plasmid. When a culture is more than 50 ml, use **double** volumes of **VP1, VP2,** and **VP3 Buffer** to ensure complete cell lysis.
	Cell pellet is not well resuspended	Do **not** add VP2 Buffer until cells are completely resuspended by vortexing or pipetting.
Low yield of plasmid DNA	Not enough bacterial cells	Ensure that bacteria have grown well (OD_{600}>1) after overnight incubation with vigorous agitation.

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