HIGH PURITY PLASMID PURIFICATION KITS WITH PREFILTERS

For the isolation of ultra-pure plasmid DNA suitable for all molecular and cellular biology applications.

High Purity Plasmid Midiprep Kits with Prefilters	Catalog No. 11475-025	25 Reactions
	Catalog No. 11475-050	50 Reactions
High Purity Plasmid Maxiprep Kits with Prefilters	Catalog No. 11476-010	10 Reactions
	Catalog No. 11476-025	25 Reactions

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Better research by design

OVERVIEW

Marligen's High Purity Plasmid Purification Kits with Prefilters* combine the features of its standard anion exchange columns with the convenience of an integrated filter unit that allows simultaneous, one-step lysate clarification and column loading. A modified alkaline/SDS procedure is used to lyse the cells and precipitate the genomic DNA (1). The lysate is then poured into a pre-packed ion-exchange column containing a unique anion exchange resin to purify plasmid DNA equivalent to two passes through CsCl gradients. The column is fitted with a unique prefilter for clarifying alkaline lysates. In one combined step, the lysate is clarified and the negatively charged plasmid DNA binds to the positive charges on the surface of the resin. The temperature, salt concentration and pH of the solutions influence binding. Under moderate salt conditions, plasmid DNA remains bound to the resin while RNA, proteins, carbohydrates and other impurities are washed off. The plasmid DNA is eluted under high salt conditions. It is then desalted and concentrated by alcohol precipitation.

The protocol can be completed in 1.5 to 2 hours. All types and sizes of plasmid DNA can be purified with the High Purity Plasmid Purification Kits with Prefilters. (Note: These kits have not been validated for purifying BAC DNA.) The columns offer a binding capacity of up to 150 µg (midipreps) and 750 µg (maxipreps). Results are dependent on plasmid copy number, plasmid type, bacterial strain, and growth conditions (e.g., medium, antibiotics, temperature, and aeration). Plasmid DNA purified using the High Purity Plasmid Purification Kits with Prefilters is suitable for all molecular and cell biology applications, including transfection, automated fluorescent DNA sequencing, manual DNA sequencing, amplification reactions, *in vitro* transcription, cloning, and labeling.

Reference

1. Birnboim, H. and Doly, J. (1979) Nucleic Acids Res. 7, 1513.

*This product is the subject of U.S. Patent No. 5,843,312 and foreign equivalents as well as other pending patent applications.

COMPONENTS

Warning: This product contains hazardous reagents. It is the end user's responsibility to consult the applicable MSDS(s) before using this product. Disposal of waste organics, acids, and bases must comply with all appropriate federal, state, and local regulations. If you have any questions concerning the hazards associated with this product, please call Marligen Biosciences, Inc. at (301) 874-4990.

	VOLUMES OF COMPONENTS			
	MIDI SYSTEM		MAXI SYSTEM	
	11475-025	11475-050	11476-010	11476-025
COMPONENT NAME	**25 RXN**	**50 RXN**	**10 RXN**	**25 RXN**
Cell Suspension Buffer [50 mM Tris-HCl (pH 8.0), 10 mM EDTA]	250 ml	500 ml	100 ml	250 ml
RNase A (20 mg in Cell Suspension Buffer)	1.5 ml	2.8 ml	650 µl	1.5 ml
Cell Lysis Solution [200 mM NaOH, 1% SDS (w/v)]	250 ml	500 ml	100 ml	250 ml
Neutralization Buffer [3.1 M potassium acetate (pH 5.5)]	250 ml	500 ml	100 ml	250 ml
Equilibration Buffer [600 mM NaCl, 100 mM sodium acetate (pH 5.0), 0.15% Triton® X-100 (v/v)]	400 ml	2 x 400 ml	300 ml	2 x 400 ml
Wash Buffer [800 mM NaCl, 100 mM sodium acetate (pH 5.0)]	2 x 400 ml	3 x 500 ml	2 x 300 ml	3 x 500 ml
Elution Buffer [1.25 M NaCl, 100 mM Tris-HCl (pH 8.5)]	130 ml	250 ml	250 ml	400 ml
TE Buffer [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]	15 ml	15 ml	15 ml	30 ml
Columns/Prefilters	25	50	10	25

Additional Materials Required

- Isopropanol
- 70% ethanol
- Nucleic Acid Purification Rack (Cat. No. 11494-010)
- Tubes appropriate for pelleting and lysing cells
- Tubes appropriate in size for collecting and precipitating plasmid DNA eluted from column
- Centrifuge and rotor capable of reaching ~15,000 x g at 4°C.

Advance Preparations

- Add RNase A to Cell Suspension Buffer according to the table below. Mix well. Place a mark on the label to indicate that RNase A has been added, **then store Cell Suspension Buffer at 4°C.** All other components are stored at room temperature.

Kit	Midi		Maxi	
Reaction number	25	50	10	25
Volume of RNase	1.4 ml	2.6 ml	550 µl	1.4 ml

- Check Cell Lysis Solution for precipitate. If necessary, warm the solution briefly at 37°C to dissolve the precipitate.

CRITICAL PARAMETERS AND PROTOCOL NOTES

General

- For optimal performance use volumes, temperatures, incubation times, and centrifugations precisely as indicated in the protocol.
- Cultures may be grown in LB medium or rich media including Superbroth, Terrific Broth, 2XYT, or other proprietary media. Cell density should be 1 - 4 A_{600} units/ml.
- Do not overload the columns. Use the recommended culture volumes as indicated in the protocol to obtain optimal yield and purity.

Important Considerations for Alkaline Lysis and Neutralization Steps

Suspension

The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain. For efficient lysis, it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Cell Suspension Buffer before use.

Lysis

Mix gently but thoroughly. Do not vortex, as this may result in shearing of genomic DNA and contamination of the plasmid DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min.

Neutralization

After addition of Neutralization Buffer, a fluffy white material should form and the lysate should become less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and SDS. The lysate should be mixed thoroughly to ensure even precipitation. If the mixture still appears to contain a gelatinous and slightly brownish material, more mixing is required to completely neutralize the solution. This is more likely to happen when large cell pellets have been processed.

Special Recommendations for Processing Large Culture Volumes

If you are processing more than 50 ml of culture for Midiprep columns or more than 200 ml for Maxiprep columns to purify low copy plasmids, we strongly recommend that you double the standard volumes of Cell Suspension Buffer (with double the RNase concentration), Lysis Buffer, and Neutralization Buffer used for preparation of the cell lysate. The buffer volumes included in the kit, however, are only sufficient for preparation of the designated number of standard preps. Therefore, additional buffers will be necessary in order to utilize all of the columns in the kit for processing large culture volumes and these may be ordered from Marligen (Catalog Number 11448-100).

HIGH PURITY MIDIPREP PROTOCOL FOR USE WITH INTEGRATED PREFILTERS

BEFORE BEGINNING: Verify that RNase A has been added to Cell Suspension Buffer and that no precipitate has formed in Cell Lysis Solution (See Advance Preparations).

1. **Column Equilibration:** Apply 15 ml of Equilibration Buffer directly into the prefilter that is inserted in the column. Allow the solution in the column to drain by gravity flow. Prepare cell lysate while the column and prefilter unit are equilibrating.

   Note: Shortly after the column has begun dripping, some drops at the outlet may appear turbid. This is normal and due to the interaction of the equilibration buffer with the resin. The turbid drops will not affect the preparation in any way.

2. **Cell Harvesting**: For high copy-number plasmids (>2 µg DNA/ml culture), pellet up to 50 ml of an overnight culture. For low copy-number plasmids (~2 µg DNA/ml culture), pellet 50 to 100 ml of an overnight culture. Thoroughly remove all medium.

3. **Cell Suspension:** Add 10 ml of Cell Suspension Buffer (containing RNase A) to the pellet and suspend the cells until the mixture is homogeneous.

4. **Cell Lysis:** Add 10 ml of Cell Lysis Solution and mix gently by inverting the capped tube five times. Do not vortex. Incubate at room temperature for exactly 5 min.

5. **Neutralization:** Add 10 ml of Neutralization Buffer and mix immediately by inverting the tube until the solution is homogeneous. When large cell pellets have been processed, more vigorous shaking may be required. However, **DO NOT VORTEX!**

6. **Column Loading:** Pour the neutralized cell lysate including all of the precipitated material into the previously equilibrated prefilter/column combination. Let the lysate run through by gravity flow until the flow stops or becomes very slow (< 1 drop per 10 seconds). Discard flow-through.

7. **Lysate Wash:** Add 10 ml of Wash Buffer to the prefilter and let drain by gravity until the flow stops or becomes very slow.

   Do not force any remaining liquid out of the Prefilter!

8. **Prefilter Removal :** As soon as the column has stopped dripping, remove the prefilter from the column and discard it.

9. **Column Wash:** Wash the column with 20 ml of Wash Buffer. Allow the solution in the column to drain by gravity flow. Discard flow-through.

10. **Plasmid DNA Elution:** Elute the DNA by adding 5 ml of Elution Buffer. Allow the solution in the column to drain by gravity flow. Do not force out remaining solution.

11. **Plasmid DNA Precipitation:** Add 3.5 ml of isopropanol to the eluate. Mix, and centrifuge the mixture at ~15,000 x g at 4°C for 30 min. Carefully discard supernatant. Wash the plasmid DNA pellet with 3 ml of 70% ethanol and centrifuge at ~15,000 x g at 4°C for 5 min. Carefully and fully pipet off the ethanol wash. Air dry the pellet for 10 min.

    NOTE: The DNA pellet is easily dislodged when washing with 70% ethanol. It is best to pipet off the ethanol wash to remove it from the pellet. This is a particular problem when the DNA pellet is very small.

12. **Purified DNA:** Dissolve the pelleted DNA in 200 µl of TE Buffer. Occasionally, insoluble particles are present. These particles do not influence the quality of DNA and can easily be removed by centrifugation in a microcentrifuge at ~12,000 x g at room temperature for 1 min. Transfer the sample to a fresh tube.

HIGH PURITY MAXIPREP PROTOCOL FOR USE WITH INTEGRATED PREFILTERS

BEFORE BEGINNING: Verify that RNase A has been added to Cell Suspension Buffer and that no precipitate has formed in Cell Lysis Solution (See Advance Preparations).

1. **Column Equilibration:** Apply 30 ml of Equilibration Buffer directly into the prefilter that is inserted in the column. Allow the solution in the column to drain by gravity flow.
2. **Cell Harvesting**: For high copy-number plasmids (>2 µg DNA/ml culture), pellet up to 100 ml of an overnight culture. For low copy-number plasmids (~2 µg DNA/ml culture), pellet 200 to 500 ml of an overnight culture. Thoroughly remove all medium.
3. **Cell Suspension:** Add 10 ml of Cell Suspension Buffer (containing RNase A) to the pellet and suspend the cells until homogeneous. For culture volumes greater than 200 ml, add 20 ml of Cell Suspension buffer.
4. **Cell Lysis:** Add 10 ml of Cell Lysis Solution. For culture volumes greater than 200 ml, add 20 ml of Cell Lysis Solution. Mix gently by inverting the capped tube five times. Do not vortex. Incubate at room temperature for exactly 5 min.
5. **Neutralization:** Add 10 ml of Neutralization Buffer and mix immediately by inverting the tube until the solution is homogeneous. For culture volumes greater than 200 ml, add 20 ml of Neutralization Buffer and mix immediately by inverting the tube until the solution is homogeneous. When large cell pellets have been processed, more vigorous shaking may be required. However, **DO NOT VORTEX!**
6. **Column Loading:** Pour the neutralized cell lysate including all of the precipitated material into the previously equilibrated prefilter/column combination. Let the lysate run through by gravity flow until the flow stops or becomes very slow (< 1 drop per 10 seconds). Discard flow-through.
7. **Lysate Wash:** Add 10 ml of Wash Buffer to the prefilter and let drain by gravity until the flow stops or becomes very slow.

   Do not force any remaining liquid out of the Prefilter!
8. **Prefilter Removal:** As soon has the column as stopped dripping, remove the prefilter from the column and discard it.
9. **Column Wash:** Wash the column with 50 ml of Wash Buffer. Allow the solution in the column to drain by gravity flow. Discard flow-through.
10. **Plasmid DNA Elution:** Elute the DNA by adding 15 ml of Elution Buffer. Allow the solution in the column to drain by gravity flow. Do not force out remaining solution.
11. **Plasmid DNA Precipitation:** Add 10.5 ml of isopropanol to the eluate. Mix, and centrifuge the mixture at ~15,000 x g at 4°C for 30 min. Carefully discard supernatant. Wash the plasmid DNA pellet with 5 ml of 70% ethanol and centrifuge at ~15,000 x g at 4°C for 5 min. Carefully and fully pipet off the ethanol wash. Air dry the pellet for 10 min.

    NOTE: The DNA pellet is easily dislodged when washing with 70% ethanol. It is best to pipet off the ethanol wash to remove it from the pellet. This is a particular problem when the DNA pellet is very small.
12. **Purified DNA:** Dissolve the pelleted DNA in 500 µl of TE Buffer. Occasionally, insoluble particles are present. These particles do not influence the quality of DNA and can easily be removed by centrifugation in a microcentrifuge at ~12,000 x g at room temperature for 1 min. Transfer the sample to a fresh tube.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Suggested Solution
Low yield of plasmid DNA	Temperature of buffers too low	Store all buffers except Cell Suspension Buffer with RNAse A at room temperature.
	Low copy-number plasmid	Increase the number of cells processed.
	Lysate at improper pH or salt concentration	Carefully remove all medium before suspending cells.
	Plasmid DNA pellet over-dried	Air dry the plasmid DNA pellet so that it will fully dissolve. Do not dry the pellet with a vacuum system.
	DNA pellet lost during ethanol wash	It is easy to lose the DNA pellet during the ethanol wash. Pipet the ethanol from the tube prior to drying.
Chromosomal DNA contamination	Genomic DNA sheared in handling	Invert tubes when adding Cell Lysis and Neutralization Buffers. Do not vortex.
Additional plasmid forms present	Plasmid DNA permanently denatured	Incubate the lysate at room temperature for a maxiumum of 5 minutes. Permanently denatured DNA will appear as a band electrophoresing just ahead of the supercoiled plasmid DNA. This material will not be digested by restriction endonucleases.
RNA contamination	Lysate at improper pH, salt concentration, or temperature for binding to column	Carefully remove all medium before suspending cells.
		Ensure that excess Neutralization Buffer is not added when neutralizing the lysate.
		Ensure that the lysate has not warmed above room temperature during the centrifugation.
	Sample left on column too long	Once the lysate has been loaded on the column or cartridge, avoid delays in processing.
	Lysate droplets remaining on walls of column at elution	Wash droplets of lysate from walls of column or cartridge when adding wash buffer.
	RNAse A digestion incomplete	Use recommended volume of Cell Suspension Buffer.
		Ensure that Cell Suspension Buffer with RNAse A is stored at 4°C and is less than 6 months old.

ACCESSORIES AND RELATED PRODUCTS	SIZE	CAT. NO.
High Purity BAC Buffer Kit	Each	11448-100
Nucleic Acid Purification Rack	Each	11494-010

Quick Reference Protocol

	Midiprep Per Reaction	Maxiprep Per Reaction
Column Equilibration		
Equilibration Buffer	15 ml	30 ml
Cell Harvesting and Alkaline Lysis		
Cell Suspension Buffer*	10 ml	10 ml
Cell Lysis Buffer*	10 ml	10 ml
Neutralization Buffer*	10 ml	10 ml
Column Loading and Elution		
Wash Buffer**	10 ml	10 ml
	Discard Prefilter	
Wash Buffer	20 ml	50 ml
Elution Buffer	5 ml	15 ml
Isopropanol	3.5 ml	10.5 ml
70% Ethanol	5 ml	5 ml
TE Buffer	200 µl	500 µl

* Additional volumes may be required for Low Copy Number plasmids (Catalog # 11448-100)

Important Information

The product you have received is authorized for laboratory research use only. The product has not been qualified or found safe or effective for human or animal diagnostic or therapeutic application. Uses other than the labeled intended use may be a violation of applicable law.

For additional product information, protocols and troubleshooting information, visit our website at www.marligen.com.

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