Vader-Trap™ High Purity Maxiprep Plasmid Purification Kit

All technical literature related to this kit is available online at www.virongy.com
Please visit our website for the most up-to-date version of this technical manual.
For questions related to this product or protocol, e-mail Virongy at: info@virongy.com

Table of Contents

1. Introduction & Description

Our Vader-Trap™ plasmid purification technology significantly improves the traditional alkaline lysis procedure for plasmid purification. Virongy's Vader-Trap™ technology uses innovative abortion particles and reagent formulations to adsorb and remove contaminants and impurities, resulting in higher yield and greater purity than conventional Maxiprep spin kits. The purified plasmid DNA is suitable for many downstream applications, such as restriction digestion, transfection, cloning, and DNA sequencing, with a greater efficiency.

The Vader-Trap™ High Purity Maxiprep Plasmid Purification Kit is designed to purify up to 500 µg of plasmid DNA from a 150-500 mL overnight bacteria culture.

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2. Product Components & Storage

PRODUCT	SIZE	CATALOG No.
Vader-Trap™ Plasmid Maxiprep Purification Kit	**(10) Preps**	VTPDNA-Maxi10
Each kit contains sufficient buffer quantities to perform 10 preps.		

Component	Volume	Before You Start
• **Buffer VT**	25 ml	
• **Buffer A**	40 ml	Add RNase A & store at 4°C
• **Buffer B**	40 ml	
• **Buffer C**	112 ml	Add 48 ml 100% ethanol
• **Buffer D**	112 ml	Add 80 ml 100% ethanol
• **Buffer VEQ**	120 ml	
• **Buffer E**	20 ml	
• **RNase A**	1 ml	Add to **Buffer A**
• DNA Binding column	10 columns	

3. Before You Begin

Materials That Will Need to be Self-Supplied

- 100% ethanol
- 50 mL conical tubes
- High speed centrifuge

Before lysing the cells and purifying your plasmid DNA, equilibrate the column with 10 mL of **Buffer VEQ**.

Before You Start

- Add 1 mL RNase A to **Buffer A**. Store at 4° C.
- Add 48 mL of 100% ethanol to **Buffer C**.
- Add 80 mL of 100% ethanol to **Buffer D**.

DNA PURIFICATION PROTOCOL

Bacterial culture volume:

150 - 250 ml overnight bacterial culture for high-copy plasmids
500 ml overnight bacterial culture for low-copy plasmids

1. Grow approximately 150 - 500 mL of bacterial culture for 18 hours in 37°C.
2. Transfer the bacterial culture into 50 ml tubes
3. Add **0.25 mL** of **Buffer VT** to 50 ml bacterial culture (1/20 the volume of bacterial culture).
4. Centrifuge at 5,000 x g for 15 min at 4°C to pellet the bacterial cells.
5. Discard the supernatant.
6. Resuspend bacterial cell pellet with **3** mL of **Buffer A**.
7. Add **3 mL Buffer B**. Invert 4-5 times to gently mix.
8. Add **12 mL** of **Buffer C**. Invert 4-5 times to mix.
9. Centrifuge at 15,000 x g for 15 min at room temperature. A compact black pellet will form.
10. Equilibrate the MaxiPrep DNA binding column by placing the column into a clean 50 mL conical tube. Add **10 mL** of **Buffer VEQ**, and then centrifuge the column assembly at 1,200 rpm for 1 min.
11. Carefully decant the supernatant (from **Step 9)**, which contains the plasmid DNA, into the DNA binding column assembly, Centrifuge at 1,200 rpm for 1 min, and discard flow-through. The plasmid DNA will bind to the column.
12. Add 16 **mL** of **Buffer D** to the midiprep DNA binding column. Centrifuge at 1,200 rpm for 1 min. Discard flow-through, and centrifuge again at 1,200 rpm for 1 min to dry the column.
13. Place Maxiprep DNA binding column into a clean 50 mL conical tube and elute plasmid DNA by adding **1 mL** of **Buffer E**. Incubate at room temperature for 1 min, and then centrifuge at 1,200 rpm for 1min. (*Optional: add* 0.5 - 1 ml **Buffer E** *to the column to elute residual DNA.*)
14. Check quality and quantity of DNA using a spectrophotometer.

4. Troubleshooting

This guide provides brief recommendations for potential problems with plasmid purification results. For additional information and assistance, contact Virongy.

Problem	Recommendation
Low plasmid DNA yield	Increase the volume of culture: 350 - 400 mL for high-copy plasmids; 600 ml for low-copy plasmids. Thoroughly resuspend cells with **Buffer A**. Wrong reagents used. Be sure to use buffers provided in the Vader-Trap™ Purification Kit and in the order instructed. Some bacteria are more resistant to cell lysis. Incubate for up to 5 minutes to allow for more efficient lysis.
Genomic DNA Contamination	Overmixing or vortexing of sample following cell lysis. After the addition of **Buffer B**, do not overmix or vortex sample.
No plasmid DNA present	Prepare all buffers as directed in section 3 (Before You Begin). Ensure that 100% ethanol is added. Column was not equilibrated with **Buffer VEQ** before cell lysis.
Presence of RNA	Verify that RNase A was added into **Buffer A**.
Poor Cell Lysis	Bacterial culture is too concentrated and excessive biomass prevents complete lysis. Bacteria cultures should be grown in LB medium with the appropriate antibiotics.

5. Related Products

Product	Size	Catalog No.
Vader-Trap™ High Purity MiniSpin Plasmid Purification Kit	60 columns	VTPDNA-60
Vader-Trap™ High Purity MiniSpin Plasmid Purification Kit	120 columns	VTPDNA-120
Vader-Trap™ High Purity MidiPrep Plasmid Purification Kit	20 columns	VTPDNA-Midi20
Vader-Trap™ High Purity MidiPrep Plasmid Purification Kit	100 columns	VTPDNA-Midi100
Vader-Trap™ High Purity MaxiPrep Plasmid Purification Kit	10 columns	VTPDNA-Maxi10
Vader-Trap™ High Purity MaxiPrep Plasmid Purification Kit	50 columns	VTPDNA-Maxi50