

# **Column-Pure™ Plasmid Mini Prep Kit**

## **Cat. No. D504**



**Catalog No.:** D504

**Product Name:** Column-Pure™ Plasmid Mini Prep Kit

**Size:** 100 preps

**Description:** The most widely used kit in modern molecular biology laboratories; this kit, utilizes a silica spin filter to purify plasmid DNA. It is the easiest method for isolation of plasmid DNA and produces high-yield plasmid DNA. The recovered plasmid DNA has a 1.8-2.0 OD<sub>260/280</sub> ratio and is ready for such downstream applications as automated sequencing, and restriction digests. The purified plasmid DNA is primarily in the supercoiled form.

<b>Kit Contents:</b>	Solution I	12ml	Elution Buffer	10ml
	Solution II	24ml	RNase A	1 vial
	Solution III	2x25ml	Spin Columns	100
	Wash Solution	2x20ml		

*\*User will supply Ethanol and 1.5 microcentrifuge tubes.*

**Storage:** Store the kit at room temperature. However, Solution I may be stored at 4°C for long-term storage. RNase A should be stored at 4°C.

**Caution:** **Solution III** contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. **DO NOT** add bleach or acidic solutions directly to waste containing these buffers.

In case of spills, clean with suitable laboratory detergent and water first, and then take proper procedures appropriate for your specific research environment.

For **Solution II and Solution III**: Always wear gloves and protective clothing; including an eye or face protector when using this kit. For all the solutions in the kit, avoid contact with skin and eyes.

Do not inhale or swallow.

Keep away from food, drink, and animal feed.

Keep out of children's reach.

In case of accidental exposure, seek immediate medical attention.

All MSDS are available on request.

## Protocol

**Note:** *Before use, transfer the RNase A to Solution I, add 80ml ethanol to the Wash Buffer bottles to make the final 1X Wash Buffer, and mix both solutions gently but well.*

1. **Collection of Bacteria:** Use a 1.5ml microcentrifuge to pellet 1-5ml overnight culture of *E. coli* in LB medium with appropriate antibiotics. Completely discard the supernatant.
2. **Resuspension:** Add 100  $\mu$ l Solution I. Fully resuspend the bacterial pellet by vortexing.
3. **Lysis:** Add 200  $\mu$ l Solution II and mix immediately but gently by inverting the microtube 4-6 times.
4. **Neutralization:** Add 350  $\mu$ l Solution III. Gently invert the microtube 4-6 times to mix and then centrifuge for 5 minutes at full speed ( $>10,000$  rpm) in a microcentrifuge.
5. **DNA Binding:** Transfer the supernatant to the Spin Column, centrifuge for 1 minute, and discard the flow-through.
6. **Wash:** Add 700  $\mu$ l Wash Buffer, centrifuge for 1 minute, and discard the flow-through.
7. **(Optional Wash):** If desired, wash the column again as in Step 6.
8. Centrifuge the column for ***one more additional minute*** to remove any residual Wash Buffer.
9. **Elution:** Transfer the column to a new 1.5ml microtube, add 50  $\mu$ l Elution Buffer to the center of the Spin Column, and centrifuge at full speed for 1 minute.

The plasmid DNA is now ready to use for any downstream applications, such as restriction digestion, transformation or even transfection.

**Related Products**

*Column-Pure™ DNA Gel Recovery Kit, Cat No. D507*

*Column-Pure™ PCR Clean-Up Kit, Cat. No. D509*

*100bp DNA Ladder, Cat. No. M107*

*1Kb DNA Ladder II, Cat. No. M108*

*Standard-Agarose, Cat. No. A113*