

Plasmid Miniprep Kit

06/16

(Catalog # K529-100; 100 isolations; Store at Multiple Temperatures)

I. Introduction:

Amplification and purification of plasmid DNA is a necessary step for numerous recombinant DNA technology applications. BioVision's Plasmid Miniprep Kit eases the purification of low and high copy plasmids from bacteria. DNA purified by this kit can be used for a variety of downstream molecular biology applications such as cloning, PCR, transfections/transformations, sequencing, enzymatic analysis etc.

II. Applications:

- PCR, cloning, transformations, transfections, restriction enzyme digest/analysis, sequencing

III. Sample Type:

- Low and high copy plasmids

IV. Kit Contents:

Components	K529-100	Cap Code	Part Number	Storage (°C)
Buffer R [Re-suspension Buffer]	25 ml	NM/Clear	K529-100-1	RT/4°C
RNAse A	250 µl	Blue	K529-100-2	-20°C
Buffer L [Lysis Buffer]	25 ml	NM/Brown	K529-100-3	RT
Buffer N [Neutralization Buffer]	35 ml	NM/Clear	K529-100-4	RT
Buffer ER [Endonuclease Removal Buffer]	34 ml	NM/Red	K529-100-5	RT
Buffer W [Wash Buffer]	15 ml	WM	K529-100-6	RT
Buffer E [Elution Buffer]	22 ml	WM	K529-100-7	RT
Spin Columns/Collection Tubes	100 tubes	-	K529-100-8	RT

V. User Supplied Reagents and Equipment:

- DNase-free aerosol tips and micro-centrifuge tubes, 100% Ethanol, Heating Block, Centrifuge

VI. Storage Conditions and Reagent Preparation:

Refer to section IV (Kit contents) for proper storage. Protect from light. Briefly centrifuge small vials prior opening. Read entire protocol before performing the assay.

- **Buffer L, N, and E:** Ready to use. Store at RT.
- **Buffer R:** Store at RT prior to RNAse A addition.
- **RNAse A:** Ready to use. Keep at -20°C for long term storage. Add the entire contents of the RNAse A tube to Buffer R, mix well, and store the entire solution at 4°C.
- **Buffer ER:** Add 21.5 ml of 100% Ethanol, molecular biology grade. Mix well and store at room temperature.
- **Buffer W:** Add 68 ml of 100% Ethanol, molecular biology grade. Mix well and store at room temperature.
- **Spin Columns:** Ready to use. Store at room temperature in dry conditions

VII. Plasmid Miniprep Protocol:

1. Bacterial Lysis:

- Transfer 1 ml of overnight bacterial culture harboring the plasmid of interest into a 1.5 ml tube and pellet the cells by centrifugation at 12,000 x g for 1 minute at 4°C. Discard the supernatant.

Note: For low copy plasmids, spin down up to 5 ml of overnight bacterial culture and proceed to step (b).

- Re-suspend the pellet in 250 µl Buffer R [Re-suspension Buffer] with added RNAse A. Do not leave clumps.
- Add 250 µl of Buffer L [Lysis Buffer] and invert 5 times to mix or until clear lysate is observed.
- Add 350 µl Buffer N [Neutralization Buffer] to the lysate and immediately mix by inverting the tube 5 times.
- Place the tube in a pre-cooled centrifuge at 4°C and centrifuge for 10 minutes at 12,000 x g.

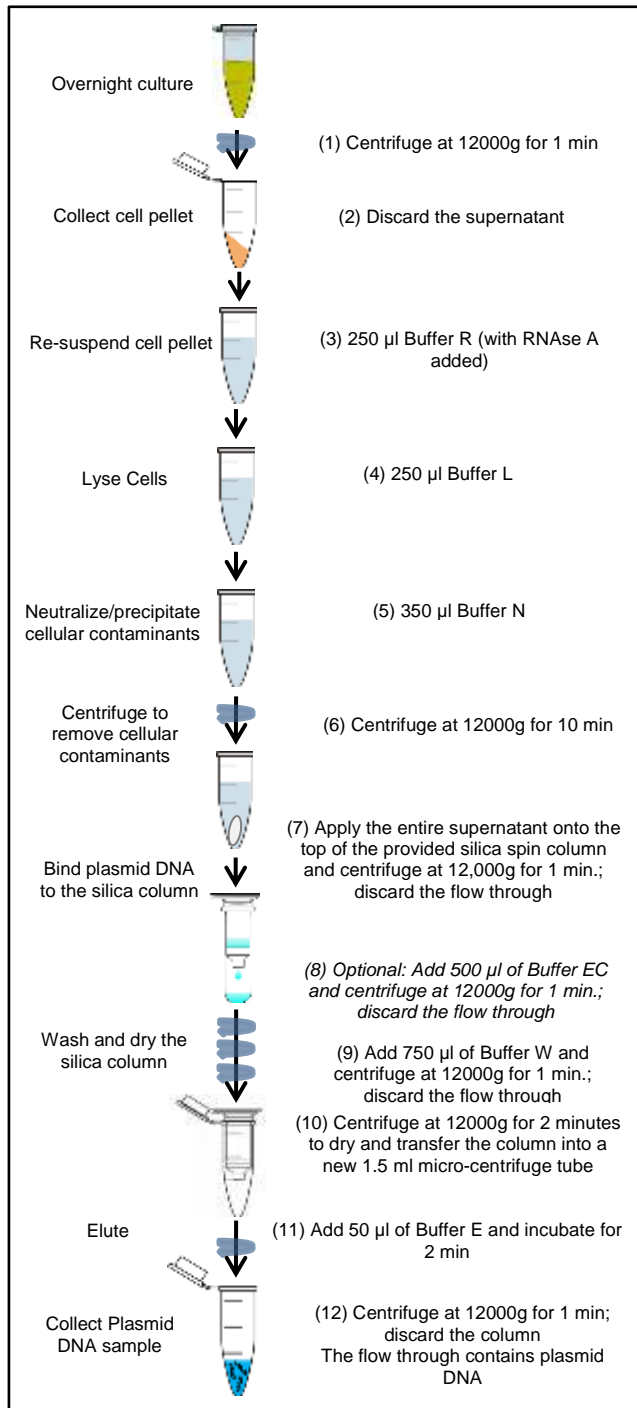
2. Column Binding and Washing:

- Place the spin column into the 2 ml collection tube (both provided with the kit).
 - Apply the entire supernatant from step "e" onto the top of the spin column.
 - Centrifuge at 12,000 x g for 1 minute at 4°C.
 - Optional Wash Step: Pipette 500 µl of Buffer ER [Endonuclease Removal Buffer] to the top of the spin column and centrifuge at 12,000 x g for 1 minute at 4°C.
- Note:** This step is required if endA+ bacterial strains containing high levels of endonuclease activity are used.
- Discard the flow through and pipette 750 µl of Buffer W [Wash Buffer] onto the top of the spin column.
 - Centrifuge at 12,000 x g for 1 minute at 4°C and discard the flow through.
 - Place the column back into the centrifuge and spin for 2 additional minutes at 12,000 x g to remove residual ethanol.

3. Plasmid Elution:

- Transfer the spin column to a clean, DNase-free 1.5 ml tube.
- Add 50-100 µl of Buffer E [Elution Buffer] to the top of the spin column and incubate for 1-2 minutes at room temperature.
- Spin at 12,000 x g for 1 minute at 4°C to elute the plasmid DNA. The flow through contains purified plasmid DNA.
- Sample is now purified and ready to use. Store plasmid DNA at -20°C or immediately use the sample in a downstream application of your choice.

VIII. Protocol Quick Guide



FOR RESEARCH USE ONLY!
Not to be used on humans

IX. Trouble Shooting

Issue	Possible Reason	Recommendations
Low yield	Low bacteria concentration	Monitor OD ₆₀₀ of your overnight culture. We recommend OD ₆₀₀ range between 1 and 2.
	The plasmid is a low copy number plasmid	Increase the number of bacterial cells used by spinning down up to 5 ml of overnight cell culture.
No DNA band/ severe smear on gel	DNAse / endonuclease contamination	Use DNase free aerosol tips, DNase-free tubes, and practice good sterile technique
		Perform the endonuclease removal wash with buffer [Buffer ER] in step "i" of the "column binding and washing" section to remove contaminating cellular endonucleases

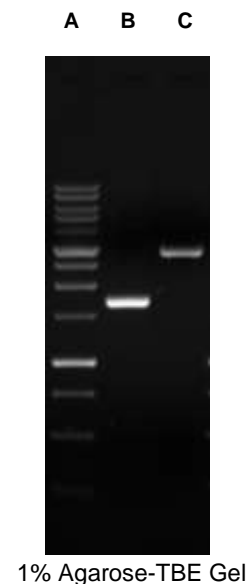


Figure 1: Isolation of pUC19 plasmid from DH5 α cells using BioVision's Plasmid Miniprep DNA Isolation Kit.
Lane A: BriteRuler 1 kb DNA Ladder (Cat#9301-100)
Lane B: pUC19 plasmid DNA, uncut/supercoiled
Lane C: pUC19 plasmid DNA, linearized with NdeI Restriction Endonuclease.