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Bacterial DNA Purification Kit II

06/20

(Catalog # K1457-50, -250; 50 or 250 Preps; Store at Multiple Temperatures)

I. Introduction:

BioVision's Bacterial DNA Purification Kit II provides a rapid and reliable method for isolating high-quality total DNA from a wide variety of bacterial species using spin columns. This kit will isolate all cellular DNA, including plasmid DNA. Up to $1x10^9$ bacterial cells can be processed using a spin column. In this kit, bacterial cells in log-phase are harvested and the bacterial cell wall is digested using lysozyme and Proteinase K. The lysed samples are applied to the spin column. The DNA efficiently binds to the spin column, while proteins and other contaminants are removed. Pure DNA is then eluted in sterile water or elution buffer. Each spin column can bind approximately 100 µg genomic DNA. DNA purified using this kit can be used for downstream applications such as PCR, restriction digestion, and hybridization techniques.

II. Application:

- To extract genomic DNA from Bacteria

III. Key Features

- Rapid, easy and convenient
- **Highly pure**, high yield
- **Many downstream applications** such as PCR, restriction digestion, and hybridization
- High quality spin columns

IV. Sample Types:

- Bacteria

V. Kit Contents:

Components	K1457-50 (50 Preps)	K1457-250 (250 Preps)	Part Number
Buffer TL	15 ml	65 ml	K1457-XX-1
Lysozyme	50 mg	250 mg	K1457-XX-2
Lysozyme Storage Buffer	5 ml	10 ml	K1457-XX-3
RNase A	100 µl	500 µl	K1457-XX-4
Glass Beads	50 x 25 mg	250 x 25 mg	K1457-XX-5
Proteinase K	1 ml	5 ml	K1457-XX-6
Buffer BL	15 ml	65 ml	K1457-XX-7
DNA Column	50	250	K1457-XX-8
Buffer KB	28 ml	135 ml	K1457-XX-9
*DNA Wash Buffer	12 ml	50 ml	K1457-XX-10
Elution Buffer	15 ml	70 ml	K1457-XX-11

DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 48 ml (K1457-50) or 200 ml (K1457-250) of 100% Ethanol to DNA Wash Buffer bottle before use. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

VI. User Supplied Reagents and Equipment:

- Pipettes, Pipette tips
- 100% Ethanol, Sterile water
- Sterile, nuclease-free 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge
- Waterbaths set at 30°C, 55°C, 65°C

VII. Shipping and Storage Conditions:

The kit is shipped in a gel pack. All reagents except Proteinase K, Lysozyme and RNase must be stored at room temperature (RT). Once reconstituted in buffer, Proteinase K and Lysozyme must be stored at -20°C in aliquots. RNase A must be stored at 4°C.The kit reagents will be stable for 12 months if stored properly.

VIII. Reagent Preparation and Storage Conditions:

1. DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 48 ml (K1457-50) or 200 ml (K1457-250) of 100% Ethanol to DNA Wash Buffer bottle before use. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Add 1 ml (K1457-50) or 5 ml (K1457-250) of lysozyme storage buffer to dissolve the lysozyme. Store the aliquots at -20°C and thaw before use.
3. Warm the Elution buffer at 65°C.
4. Buffer BL and Buffer KB contains chaotropic salts, which may form reactive compounds with bleach. Do not add bleach or acidic solutions directly to the preparation waste. Wear gloves and protective eyewear when handling this buffer.
5. A precipitate may form in Buffers BL and TL under cool ambient conditions. Warm the bottle at 37°C to dissolve the precipitate before use. Store these buffers at RT.
6. All centrifugation steps can be carried out at RT.

IX. DNA Purification:

This protocol is designed to purify genomic DNA from a sample volume of 1-3 ml of Bacterial cultures in log phase of growth in LB medium. Overnight culture can be used in many cases.

1. Pellet 1- 3 ml of **Bacterial culture** by centrifugation at 12,000 x g for 2 min at RT.
2. Discard the medium completely and resuspend the pellet in 180 µl **Buffer TL**. Add 18 µl of **lysozyme solution** and 2 µL **RNase A**, incubate at 30°C for 15-30 min.

Note: Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield more genomic DNA.

3. Centrifuge the cells at 5,000 x g for 5 min at RT. Discard the supernatant and leave **10 µl residual liquid** supernatant with the cells in the tube. Resuspend the cell pellet by vortexing.
4. Add 25 mg **glass beads** and 200 µl **Buffer TL**. Vortex at maximum speed for 1 min. Allow the beads to settle down to the bottom of the tube and transfer supernatant to a new 1.5 ml centrifuge tube.
5. Add 20 µl **Proteinase K** and vortex for 10 sec. Spin down briefly.
6. Incubate the mixture at 55°C for 10 min.
7. Add 220 µl **Buffer BL** and briefly vortex to mix. Incubate at 65°C for 10 min. A wispy precipitate may form upon addition of Buffer BL (The precipitate does not interfere with DNA recovery).
8. Add 220 µl **100% ethanol** and mix thoroughly by vortexing for 20 sec. If any precipitate can be seen at this point, break the precipitates by pipetting up and down 10 times.
9. Transfer the entire sample from Step 9 into a **DNA column**, including any precipitate that may have formed. Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the flow-through liquid.
10. Place the DNA column back in the 2 ml microcentrifuge tube and add 500 µl of **Buffer KB** into the DNA column. Centrifuge at 10,000 x g for 1 min. Discard the flow-through liquid.
11. Place the DNA column in the same microcentrifuge tube and wash by adding 500 µl **DNA Wash Buffer**. Centrifuge at 10,000 x g for 1 min. Discard the flow-through liquid and place the column back in the microcentrifuge tube.
12. Centrifuge at 12,000 x g for 2 min to dry the column.
13. Place the column in a 1.5 ml microcentrifuge tube and add 50-100 µl **Elution Buffer** and centrifuge at 10,000 x g for 1 min to elute the DNA.
    Optional: Adding the eluted DNA back to the column for a second elution will yield another 20-30% of the DNA while the first elution typically yields 60-70% of the DNA.

X. General Troubleshooting Guide:

Problem	Possible Cause	Suggestions
Column cogged	Sample is too large	Do not use greater than 3 ml of culture at OD_{600} = 1.0 or 1 x 10^9 bacterial cells per spin column. For larger volumes, divide the samples into multiple tubes.
	Incomplete lysis	Add the correct volume of Buffer TL and incubate at 55°C for complete lysis. It may be necessary to extend the incubation time to 30 min.
	Cell remnants	Add more lysozyme or increase the incubation time for lysis. It may be necessary to increase the incubation time to 30 min.
Low DNA yield	Clogged column	See above.
	Poor elution	Repeat elution or increase the elution volume. Incubating the column at 65°C for 5 min after addition of Elution Buffer may increase the yield.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation. It will not interfere with PCR or restriction digests.
	Incomplete mixing with Buffer BL	Repeat the procedure. Make sure to vortex the sample with Buffer BL immediately and completely.
	Insufficient incubation	Increase the incubation time with Buffer TL. Ensure that no visible cell clumps remain.
	Trace protein contamination	Following step 10, wash the column with 300 µl Buffer KB before proceeding to step 11.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer TL	Mix thoroughly with Buffer BL and incubate at 70°C prior to adding ethanol.

XI. Related Products:

BioVision Product Name	Cat. No.	Sizes
ExoDNAPS™ Circulating and Exosome-associated DNA Extraction Kit (Human Plasma/Serum, 20 reactions)	K1230-20, -40	20, 40 Reactions
ExoDNAUC™ Circulating and Exosome-associated DNA Extraction Kit (Urine/Cell Media, 20 reactions)	K1231-40	40 Reactions
Soil Genomic DNA Kit	K1411-50, -250	50, 250 Preps
Yeast Genomic DNA Kit	K1414-50, -250	50, 250 Preps
Fungal Genomic DNA Kit	K1415-50, -250	50, 250 Preps
Buccal Swab DNA Purification Kit	K1466-50, -250	50, 250 Preps
Eye, Nose & Swabs gDNA Purification Kit	K1467-50, -250	50, 250 Preps
Saliva DNA Purification Kit	K1468-50, -250	50, 250 Preps
Bacterial Genomic DNA Isolation Kit	K309	100 Preps

FOR RESEARCH USE ONLY! Not to be used on humans.