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MagPure™ 96-well Plasmid Purification Kit

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(Catalog # K1477-1, -4; 1 or 4 Plates; Magnetic Beads based purification; Store at Multiple Temperatures)

I. Introduction:

BioVision's MagPure™ 96-well Plasmid Purification Kit combines the efficiency of magnetic bead technology with alkaline-SDS lysis of bacterial cells to purify high quality plasmid DNA. The kit is used to extract and purify plasmids using a 96-well Lysate Clearance Plate and magnetic particles. The cleared lysate obtained using the 96-well clearance plate in the first step is further purified using magnetic beads. The proprietary magnetic particles bind to DNA or RNA avidly, but reversibly under optimal conditions. The new 96-well Lysate Clearance Plate removes the need of time-consuming centrifugation to clear bacterial alkaline lysates. Proteins and other contaminants are removed and the nucleic acids can then be easily eluted with deionized water or low salt buffer. The purified plasmid can be used directly for automated fluorescent DNA sequencing and other standard molecular biology techniques such as restriction enzyme digestion**.** 1 ml of overnight culture in LB medium typically yields 10-15 µg high-copy plasmid DNA.

II. Application:

- Parallel purification of plasmids from 96 samples

III. Key Features:

- Rapid, easy and convenient
- **Highly pure DNA**, high yield
- **Many downstream applications** such as DNA Sequencing, Restriction Enzyme digestion etc.
- **Highly efficient Magnetic particles**

IV. Sample Types:

- Bacterial cells

V. Kit Contents:

Components	K1477-96-1 (1 pack) 96 preparations	K1477-96-4 (4 packs) 96 x 4 preparations	Part Number
Sealing film	4	16	K1477-96-X-1
Buffer A1	15 ml	70 ml	K1477-96-X-2
*RNaseA	100 µl	250 µl	K1477-96-X-3
Buffer B1	15 ml	70 ml	K1477-96-X-4
Buffer N1	15 ml	70 ml	K1477-96-X-5
96-well Lysate Clearance Plate	1	4	K1477-96-X-6
MagPure Particle Solution	1 ml	5 ml	K1477-96-X-7
**DNA Wash Buffer	12 ml	50 ml	K1477-96-X-8
Elution Buffer	15 ml	50 ml	K1477-96-X-9
96-well Binding Plate (500 µl)	1	4	K1477-96-X-10

**Add the vial of RNase A to a bottle of Buffer A1. **DNA Wash Buffer must be diluted with 100% Ethanol before starting. Add 48 ml (K1477-96-1) or 200 ml (K1477-96-4) 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, 80% Ethanol
- DD Water
- Centrifuge with swinging-bucket rotor for plates
- 500 µl plate or 1.2 ml round well plate
- 96-well 2 ml culture plate
- Multiple channel pipettor
- Magnetic Separation Stand for plates

VII. Shipping and Storage Conditions:

The kit is shipped in a gel pack. All reagents except the Buffer A1 containing RNase A and MagPure Particle Solution must be stored at room temperature (RT). Buffer A1 containing RNase A and MagPure Particle Solution should be stored at 4°C. The kit reagents are stable for 12 months if stored as recommended.

VIII. Reagent Preparation and Storage Conditions:

1. Add the vial of RNase A to a bottle of Buffer A1 and store at 2-8°C.
2. DNA Wash Buffer must be diluted with 100% Ethanol before starting. Add 48 ml (K1477-96-1) or 200 ml (K1477-96-4) 100% Ethanol to DNA Wash Buffer bottle before use.

IX. DNA Purification Protocol:

1. Culture volume: **Inoculate** 1.0-1.5 ml LB/antibiotic medium in a 96-well 2 ml culture plate (not provided) and grow bacteria at 37°C with agitation plate/block for 16-20 h. Note: It is strongly recommended that an endA negative strain of *E.coli* such as DH5α, Top 10 etc. be used for routine plasmid isolation.
2. **Seal** the plate with Sealing Film and pellet bacteria by **centrifugation** at 3000 x g in a swinging-bucket rotor for 10 min at RT.
3. Remove the film and discard the supernatant into a waste container. Dry the plate by placing upside-down on a paper towel to remove the excess media. Add **150 µl of Buffer A1 containing RNase A** to the bacterial pellet in each well of the 96-well plate. Resuspend the cells completely by pipetting. Note: Complete re-suspension of the cell pellet is vital for obtaining good plasmid yields.
4. Add 150 µl of **Buffer B1** and mix by shaking and rotating the plate for 2 min to obtain a clear lysate. Incubate at RT for 5 min. Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity.

Add 150 µl of **Buffer N1** and mix by shaking and rotating the plate for 2 min until a flocculent white precipitate forms. Incubate the sample at RT for 5 min. Note: It is critical to mix the solution well. If the mixture still appears conglobated, brownish or viscous, more mixing is required to completely neutralize the solution.

5. Clear the cell lysates with centrifugation. Place the **96-well Lysate Clearance Plate** (provided) on top of a 1.2 ml round well plate (not provided) and add the cell lysate to the Lysate Clearance Plate. Allow the cell lysate to stand for 5 min. The white precipitate should float to the top. Centrifuge at 2,000 x g for 5 min. Discard the 96-well Lysate Clearance Plate.
6. Add 10 µl of **MagPure Particle Solution** (beads) per well to the clear lysate in the round well plate. Mix well by pipetting few times. Note: *The MagPure Particle Solution contains magnetic beads that settle at the bottom of the container. Gently shake or vortex the container until the beads have re-dispersed in solution.
7. **Incubate** for 5 min at RT, mix once by pipetting or briefly vortexing. Use fresh tips to avoid cross-contamination. Note: For low copy number plasmid insolation, a 25 min or overnight incubation can increase the yield at RT.
8. Place the plate on the **Magnetic Separation Stand** (not provided) and remove the supernatant after the magnetic particles have completely migrated to the walls of each well adjacent to the magnets. Note: Supernatant should be clear when the migration is complete.
9. Remove the plate from the Magnetic Separation Stand and add 300 µl of **DNA Wash Buffer**. Resuspend the particles in DNA Wash Buffer by pipetting or gently vortexing the plate. Place the plate on the Magnet Separation Stand again and remove the supernatant after the magnetic beads have completely migrated to the walls of the plate.
10. Remove the plate from the Magnetic Separation Stand and add 300 µl of **80% Ethanol**. Resuspend the particles by pipetting. Again place the plate on the Magnet Separation Stand and remove the supernatant after magnetic beads have completely migrated to the walls of the plate.
11. **Air dry** the magnetic beads pellet for 20-30 min at RT. Remove the plate from the Magnetic Separation Stand.
12. Elute the DNA by resuspending the magnetic beads in 50-100 µl of **Elution Buffer**.
13. Place the plate onto the Magnetic Separation Stand to pellet the Magnetic beads.
14. Transfer the **supernatant** containing the purified plasmid into a clean 96-well plate (not supplied) after magnetic beads have completely migrated to the walls of the plate.

X. General Troubleshooting Guide:

Problem	Possible Reason	Suggested Improvement
Low DNA yield	Poor cell lysis	Do not use more than 1 ml of the sample for high copy plasmids.
		Cells may not be dispersed adequately prior to addition of Buffer A1. Vortex the cell suspension to completely disperse the cells.
		Increase the incubation time with Buffer B1 to obtain a clear lysate.
		If Buffer B1 is not tightly closed, it may need to be replaced using 0.2 N NaOH, 1% SDS.
	Bacterial culture overgrown or not fresh	Do not incubate the cultures for more than 16 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
	Low copy-number was plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 ml overnight culture.
DNA did not elute with the elution buffer	DNA Wash Buffer or Binding Buffer was not diluted with absolute ethanol	Prepare the DNA Wash Buffer as instructed in the datasheet.
High molecular weight DNA contamination of product	Excessive mixing of cell lysate upon addition of Buffer B1	Do not vortex or mix aggressively after adding Buffer B1. Adequate mixing is attained by simply inverting and rotating tube to cover walls with viscous lysate.
Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column may increase A260	Make sure to wash the Mag-Bind pellet as instructed. Alternatively, use agarose gel/ethidium bromide electrophoresis for quantification.
RNA visible on agarose gel	RNase A not added to Buffer A1	Add 1 vial of RNase to each bottle of Buffer A1.
Plasmid DNA floats out of well while loading agarose gel	Ethanol was not completely removed before elution	Increase the air dry time before the elution step
Plasmid DNA will not perform in downstream application	Traces of ethanol remain on the plate prior to elution	The DNA plate must be washed with absolute ethanol and dried before elution. Ethanol precipitation may be required following elution.

XI. Related Products:

BioVision Product Name	Cat. No.	Sizes
96-well Plasmid ezFilter Mini Kit	K1325	1, 4 Plates
Plasmid Miniprep Kit I	K1312	50, 250 Preps
Plasmid Miniprep Kit II	K1313	50, 250 Preps
Plasmid ezFilter Midi Kit I, Centrifuge	K1316	2, 10, 25 Preps
Plasmid ezFilter Midi Kit II, Centrifuge	K1317	2, 10, 25 Preps
Plasmid ezFilter Maxi Kit	K1319	10, 25 Preps
Plasmid ezFilter Mega3 Kit	K1320	1, 2, 10 Preps
Express Plasmid Midiprep Kit (25 min)	K1323	2, 10, 25 Preps
Plasmid Miniprep Kit	K529	100 Preps

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