Lumiprobe
Life science solutions

LumiSpin® PLASMID, Plasmid DNA Isolation Spin Kit manual

Contents

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LumiSpin® PLASMID, Plasmid DNA Isolation Spin Kit manual

The kit allows rapid (about 15 minutes) and highly efficient purification of plasmid DNA (up to 30 µg) from *Escherichia coli* cultures using spin columns. The purified DNA is suitable for any subsequent application, such as PCR, restriction enzyme digestion, ligation and transformation, sample preparation for Sanger sequencing and NGS, etc.

Kit components

Kit component	Count		
	11583 10 minipreps	**21583 50 minipreps**	**31583 100 minipreps**
G1250, Resuspension Solution, 5 mL	1	—	—
11650, RNase A (10 mg/mL), 50 uL	1	—	—
D1450, Neutralization Buffer, 2 mL	2	—	—
H2450, Wash Solution A (with GuHCl), 6 mL	1	—	—
D1350, Elution Buffer (10 mM Tris-HCl, pH 8.5), 1.5 mL	1	—	—
13164, Spin column (up to 30 µg), 10 pcs	1	—	—
M1250, Resuspension Solution, 15 mL	—	1	—
31650, RNase A (10 mg/mL), 150 uL	—	1	—
M1450, Neutralization Buffer, 20 mL	—	1	—
P2450, Wash Solution A (with GuHCl), 30 mL	—	1	—
K1350, Elution Buffer (10 mM Tris-HCl, pH 8.5), 10 mL	—	1	—
P1250, Resuspension Solution, 30 mL	—	—	1
51650, RNase A (10 mg/mL), 300 uL	—	—	1

P1550, Lysis Solution, 30 mL	—	—	1
R1450, Neutralization Buffer, 40 mL	—	—	1
T2450, Wash Solution A (with GuHCl), 60 mL	—	—	1
M2250, Wash Solution B (Concentrate to be diluted 5x with ethanol 96%), 20.0 mL	—	—	2
M1350, Elution Buffer (10 mM Tris-HCl, pH 8.5), 20 mL	—	—	1
M1550, Lysis Solution, 15 mL	1	1	—
K2250, Wash Solution B (Concentrate to be diluted 5x with ethanol 96%), 10.0 mL	1	2	—
Collection tube for spin column, 2 mL	10	50	100
23164, Spin column (up to 30 µg), 50 pcs	—	1	2

Store and transport at room temperature.

Shelf life 12 months.

Equipment and reagents supplied by user:

- microcentrifuge with rotor for 1.5 mL tubes and capable of centrifuging >10,000 RPM (6,700 × *g*);
- 96 % ethanol;
- 1.5 mL microcentrifuge tubes (2 tubes for DNA purification from 1 biological sample);
- (optional) tabletop centrifuge with rotor for 15 mL tubes and capable of centrifuging >4,500 × *g* for centrifugation of overnight bacterial culture (alternatively, a microcentrifuge with rotor for 1.5 mL tubes can be used).

Before starting

1. Transfer the contents of *RNase A* vial into the *Resuspension Solution* vial and mix thoroughly. Mark on the label that *RNase A* has been added.
2. Dilute the concentrate of *Wash Solution B* 5× with 96 % ethanol (add 4 volumes of 96 % ethanol to 1 volume of concentrate specified on the bottle). Mark on the label that ethanol has been added.
3. If any precipitate has formed in *Lysis Solution, Neutralization Buffer, Wash Solution A*, incubate the solutions at a temperature not higher than 50 °C until the precipitates have been completely dissolved. Before use let the solutions cool down to 25 °C.

Purification of plasmid DNA

All centrifugation steps are carried out at room temperature, at 10,000–13,000 RPM (6,700–11,000 × *g*) (unless specified otherwise).

Process bacterial cultures with OD_{600} no more than 30 AU (for instance, 15 mL of the culture with OD_{600} = 2 AU). If a larger cell amount should be processed, volumes of *Resuspension Solution, Lysis Solution, Neutralization Buffer* should be increased proportionally, and the supernatant should be loaded to the column in several stages.

1. Centrifuge 2.5–7 mL of *E. coli* overnight culture (10–15 mL for low-copy-number plasmids) for 5 minutes, 5,000 RPM (4,500 × *g*) or for 1 minute, 13,000 RPM (10,000 × *g*). Remove the supernatant and all remaining culture medium.
2. Resuspend the cell pellet in 250 µL of *Resuspension Solution*. Transfer the cell suspension into a clean 1.5 mL microcentrifuge tube.
3. Add 250 µL of *Lysis Solution,* mix gently by inverting the tube 4–6 times. Check that a turbid solution has become viscous and clear, and immediately proceed to the next step.

   ! In case of processing 10–15 mL of bacterial culture, mix thoroughly by inverting the tube until the solution has become viscous and clear. In some instances, more inversions may be required.

   ! Do not vortex the sample. This can cause shearing of chromosomal DNA.
4. Add 350 µL of *Neutralization Buffer* to the sample, mix gently by inverting the tube 4–6 times. As a result precipitations in the form of flakes should appear in the sample.

   ! In case of processing 10–15 mL of bacterial culture, after the tube contents has been mixed, shake the tube for a short period of time (a few seconds).
5. Centrifuge the sample for 5 minutes.
6. Place a spin column in a collection tube. Carefully transfer the supernatant to the column. Centrifuge the column for 30 seconds. Discard the flow-through and place the column back into the same collection tube.

! (optional) To remove endotoxins or trace nuclease activity (when working with EndA+ strains) add 500 µL of Wash Solution A to the column (this step can result in decreasing of DNA yield by up to 20 %). Centrifuge the column for 30 seconds. Discard the flow-through and place the column back into the same collection tube.

7. Add 500 µL of *Wash Solution B*, centrifuge the column for 30 seconds. Discard the flow-through and place the column back into the same collection tube.
8. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the collection tube with the flow-through.
9. Place the spin column into a clean 1.5 mL microcentrifuge tube. Add 50–100 µL of *Elution Buffer* to the center of the spin column membrane. Incubate at room temperature for 1 minute. Centrifuge the column for 2 minutes. The microcentrifuge tube contains the purified DNA.

   ! For increased DNA concentrations, use a low volume of Elution Buffer. Elution with volumes of less than 50 µL is not recommended because this can be insufficient to entirely wet the membrane resulting in low DNA recovery. For increased DNA yield, use a higher volume of Elution Buffer (100 µL).

   ! If necessary, DNA can be eluted with deionized water.

Note

When measuring DNA concentration, dilute the sample only with TE buffer pH 8.5 or *Elution Buffer* supplied with the kit. Otherwise, DNA concentration and purity (OD_{260} and OD_{260}/OD_{280}, respectively) can be estimated incorrectly.

Storage of purified DNA: for long-term storage -20 °C, for short-term storage 4 °C.

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