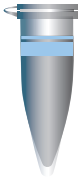
SMARTPURE Protocol

Plasmid kit

SK-PLPU-100

Sample Preparation



1. Add 1-1.5 mL overnight **bacteria culture** to 1.5 mL microcentrifuge tube.
2. Centrifuge at 10 000 × g for 30 sec
3. Discard the supernatant.

- Repeat these steps to collect more cells.

Cell Lysis

4. Resuspend completely the pellet of bacterial cells in 250 µL of **Resuspension Buffer.**
5. Add 250 µL **Lysis Buffer** and gently invert the tube 4-6 times to mix.

- Do not allow this step for more than 5 min
- Please close the bottle of the Lysis buffer immediately after usage to avoid acidification
- Do not vortex to avoid shearing of genomic DNA.

Neutralization

6. Add 350 µL of **Neutralization Buffer** and mix by gently inverting the tube 4-6 times. The solution should become cloudy and no local precipitate should be visible.
7. Centrifuge 10 min at >14 000 × g until a compact white pellet form.

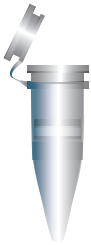
Binding

8. Place a SmartPure column into a collection tube and apply the **supernatant** to the SmartPure column
9. Centrifuge for 30-60 sec at 6 000 × g.
10. Discard the flow-through.

Washing

11. Add 650 µL of **Wash Buffer** to the Smart Pure column.
12. Centrifuge for 30-60 sec at 12 000 × g.
13. Discard the flow-through.
14. Repeat washing step once.
15. Centrifuge for an additional 1 min at 12 000 × g to remove residual liquid

Elution



16. Transfer the SmartPure column to a sterile 1.5 mL micro centrifuge tube.
17. Add 50 µL of **Elution Buffer**, ddH_2O or TE Buffer to the SmartPure column and let it stand for 1 min at room temperature.

Purified DNA

18. Centrifuge for 1 min at 12 000 × g to recover the purified plasmid DNA in the microcentrifuge tube.

- The purified plasmid DNA can be used directly or stored at -20°C for long term storage.

The SmartPure Plasmid Kit ensures an easy, fast and effective purification of up to 40 µg Plasmid DNA from 1-5 mL overnight culture of *E. coli*.

First use

→ Add all the RNase solution to the SmartPure Resuspension Buffer, mix and store at 2~8°C.

→ Add required ethanol to the SmartPure Wash Buffer (see label on the bottle) and mix well.

→ If precipitates appear in the SmartPure Lysis Buffer and the Neutralization Buffer, please incubate the solutions at 37°C for several minutes.

Kit components (100 preps)

Component	Amount
SmartPure Resuspension Buffer	25 mL
SmartPure Lysis Buffer	25 mL
SmartPure Neutralization Buffer	35 mL
SmartPure Wash Buffer	2 x 15 mL
SmartPure Elution Buffer	20 mL
RNase solution	1
SmartPure Column	100

Material not supplied

→ Sterile 1.5 mL microcentrifuge tubes
→ 10/100/1000 µL tips
→ Microcentrifuge (14 000 x g)
→ Vortex mixer
→ Absolute ethanol

Shipping & Storage conditions

The SmartPure Plasmid is shipped at room temperature. The Kit is stable for 18 months if stored in a dry environment at room temperature (15°C-25°C).

The RNase solution must be stored at 2-8°C.
After addition of the RNase solution, the SmartPure Resuspension buffer must be stored at 2-8°C

Troubleshooting

No plasmid recovery

Please check whether the ethanol had been added to the Wash Buffer bottle.

Low plasmid yield

1] Please make sure that the cell culture is fresh and cell density is optimized
2] Please ensure that the bacteria pellet is completely resuspended in the Resuspension buffer and adequately mixed.
3] Incubating Elution Buffer at 30~60°C may increase elution yields.
4] Please note that low copy number plasmids and large insert give lower yield.

Electrophoresis problem

1] Presence of Genomic DNA: the sample was mixed too vigorously in the Lysis Buffer. Invert the tubes gently 5-6 times when Lysis Buffer and Neutralization Buffer are added.
2] Presence of RNA: Add RNase A to the Resuspension buffer (100 µg/mL).

Absorbance problem

Please use the Elution Buffer to adjust zero value and dilute the sample.

→ **If the OD260/OD230 ratio is low:** wash the SmartPure column one more time.

→ **If the OD260-320/OD280-320 ratio is low:** there is a protein contamination. Please add Neutralization Buffer and centrifuge the mixture with a sufficient rotating speed to compact the precipitate. Be careful to pipette the supernatant only.

→ **If the OD260-320/OD280-320 ratio is high:** add RNase A to the Resuspension Buffer (100 µg/mL).

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