

QuickPure Plasmid Mini Kit

Item No. Q665720

Storage conditions: room temperature (15-30°C)

Product content

Component	Q665720-200T
Buffer L2	25m1
Buffer N3	80m1
Buffer PB	35m1
Buffer PW (concentrate)	25m1
Buffer EB	30m1
RNase A (10mg/ml)	800 μ1
Spin Columns DM with Collection Tubes	200

Product Introduction

The biggest feature of this kit: simple and fast, high extraction volume. The whole extraction process does not take more than 10 minutes, without centrifugation to collect bacteria and resuspend the bacterium, directly add the unique super lysate Buffer L2 to the cultured bacterial solution, followed by neutralization, centrifugation and passing through the column, and the extracted plasmid can be as high as 30 $\mu\,\mathrm{g}$, and maximize the removal of proteins, genomes and other impurities. The extracted plasmid DNA can be directly used for bacterial transformation, digestion, PCR, in vitro transcription, sequencing and other downstream experiments.

Self-contained reagent: anhydrous ethanol.

Pre-experiment Preparation and Important Notes

- 1. The kit can be stored in a dry, room temperature (15-30 $^{\circ}$ C) environment for 1 year. For longer storage, the centrifuge columns can be placed at 2-8 $^{\circ}$ C.
- 2. Before the first use, add all of the RNase A solution to Buffer N3, mix well, and store at $2-8^{\circ}$ C.
- 3. Anhydrous ethanol should be added to Buffer PW before the first use according to the instructions on the reagent bottle label.
- 4. If there is any precipitation in Buffer L2 before use, please put it in a 37° C water bath and keep mixing until the solution becomes clear before use.

Operation steps

- 1. Take 600 µl of bacterial culture into a 1.5 ml centrifuge tube (supplied).
- 2. Add 100 μ l of Buffer L2 to the above centrifuge tube and gently turn the solution up and down 8 times; the solution should change from turbid to a clear purple color, indicating complete lysis. The cleavage time should not exceed 2 minutes.
- 3. Add 350 μ l of Buffer N3 to the above centrifuge tube (please check that RNaseA has been added first) and immediately mix well by turning up and down about 8-10 times, at which point the solution should turn completely yellow and a yellow precipitate should form. centrifuge at 13,000 rpm for 2-3 minutes.



- 4. Slowly pour the supernatant obtained in step 3 into the prepared adsorption columns (Spin Columns DM with Collection Tubes) to avoid sedimentation into the columns.
- 5. Centrifuge at 13,000 rpm for 15 seconds, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
- 6. Add 150 μ 1 Buffer PB to the adsorption column and centrifuge at 13,000 rpm for 15 seconds.
- 7. Add 400 μ 1 Buffer PW to the adsorption column (please check that anhydrous ethanol has been added first) and centrifuge at 13,000 rpm for 1 minute.
- 8. Place the adsorbent column in a new centrifuge tube (self-provided), add 30-100 μ l Buffer EB to the middle part of the adsorbent membrane, centrifuge at 13,000 rpm for 1 min, collect the plasmid DNA, and store at -20° C for long term storage.

When the amount of extracted bacterial liquid is $>600 \,\mu$ l, the following procedure can be used:

- 1. This kit can extract up to 3ml of bacterial solution, if the amount of extracted bacterial solution is more than $600\,\mu\,l$, it is necessary to centrifuge the bacterial solution exceeding $600\,\mu\,l$ at 13,000rpm for 30 seconds (to collect the bacterial body), discard the supernatant and then add $600\,\mu\,l$ of bacterial solution, and then resuspend the bacterial body at the bottom of the tube thoroughly and then proceed to the following operation.
- 2. Add $100\,\mu\,l$ Buffer L2 to the above centrifuge tube, gently invert the solution up and down 10 times, if the solution is not clarified, need to continue to invert the mixing until the solution becomes a clear purple color, the lysis time should not be more than 2 minutes. (If the solution is still turbid, the amount of bacteria is too large, and the amount of bacteria should be reduced appropriately.)
- 3. Add 350 μ l of Buffer N3 to the above centrifuge tube (please check that RNaseA has been added first) and immediately mix well by turning up and down until the purple solution turns completely yellow and a yellow precipitate is formed before proceeding to the next step. centrifuge at 13,000 rpm for 5 minutes.
- 4. Transfer the supernatant to a new centrifuge tube, add 200 $\mu 1$ of isopropanol, mix up and down several times, mix well and transfer to the adsorbent column (Spin Columns DM with Collection Tubes), due to the amount of solution is too large, this time, it is necessary to centrifuge the column in two separate times, centrifugation at 13,000 rpm for 15 seconds, pour off the waste liquid in the collection tube, and put the adsorbent column back to the The adsorbent column should be placed back into the collection tube.
- 5. Add 150 μ 1 Buffer PB to the adsorption column and centrifuge at 13,000 rpm for 15 seconds.
- 6. Add 400 μ 1 Buffer PW to the adsorption column (please check that anhydrous ethanol has been added first) and centrifuge at 13,000 rpm for 1 minute.
- 7. Place the adsorbent column in a new centrifuge tube (self-provided), add 50-200 μ 1 Buffer EB to the middle part of the adsorbent membrane, leave it at room temperature for 2 min, centrifuge at 13,000 rpm for 1 min, collect the plasmid DNA, and store it at -20° C for a long time.