MCLAB
Molecular Cloning Laboratories

Manual

Version 4.1

Product name: Mini Plus Ultrapure Plasmid Prep Kit

Cat #: PPMC-100, PPMC-200

Description:

Mini Plus Ultrapure Plasmid Prep Kit provides high plasmid DNA yields. Up to 40µg of high quality plasmid DNA can be extracted using a modified alkaline lysis procedure that is combined with simple binding and wash steps. The final plasmid DNA is compatible with many downstream molecular biology applications.

Application:

Mini Plus system provides reproducible yields of high-purity DNA suitable for many applications including:
- Restriction digestion
- Transformation
- PCR
- Automated fluorescent and radioactive sequencing
- Library screening or large-scale screening

Contents:

Cat #	PPMC-100	PPMC-200
Preps	50	250
P1 Buffer	20 ml	70 ml
P2 Buffer	20 ml	70 ml
N3 Buffer	30 ml	140 ml
PB Buffer	30 ml	150 ml
PE Buffer (Ethanol added)	100 ml	500 ml
EB Buffer	15 ml	55 ml
RNase A	200 µl	700 µl
Column	50	250
Collection Tube	50	250

Shipping & Storage:

Kits should be stored dry at room temperature (15-25°C). Kits can be stored for up to 12 months without any reduction in performance and quality. For longer storage keep kits at 2-8°C. If any precipitate forms in the buffers during storage at 2-8°C, simply warm the buffers to 37°C prior to use. After addition of RNase A, Buffer P1 is stable for 6 months when stored at 2-8°C. RNase A stock solution can be stored for up to 2 years at room temperature.

Important Notes:

Buffer notes:

- Add the provided RNase A solution to Buffer P1, mix and store at 2-8°C.
- Check Buffers P2 and N3 before use for salt precipitation. Dissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification from CO_2 from the air.
- Buffers P2, N3 and PB contain irritants. Wear gloves when handling these buffers.
- RNase A is provided as a 10 mg/ml solution.

Centrifugation notes:

- All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a conventional, table-top micro centrifuge.

Protocol:

Use the micro-centrifuge procedures for purification of up to 20 µg of high-copy plasmid DNA from 1-5 ml overnight cultures of ***E. coli*** in LB medium. All protocol steps should be carried out at room temperature.

Procedure:

1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a micro centrifuge tube. Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
2. Add 250 µl Buffer P2 and gently invert the tube 4-6 times to mix. Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.
3. Add 350 µl Buffer N3 and invert the tube immediately but gently 4-6 times. To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.
4. Centrifuge for 10 min. at 13,000 rpm (~17,900 x g) in a table-top micro centrifuge. A compact white pellet will form.
5. Apply the supernatant from step 4 to the column by decanting or pipetting.
6. Centrifuge for 30-60s. Discard the flow-through.
7. Wash the spin column by adding 0.5 ml Buffer PB and centrifuging for 30-60s. Discard the flow-through. This step is necessary to remove trace nuclease activity when using end A+ strains such as JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content.
8. Wash the spin column twice with 1ml of PE Buffer by centrifuging for 30-60 seconds. Discard the flow-through. Ensure that ethanol has been added into PE Buffer prior to use.
9. Discard the flow-through, and centrifuge for an additional 1 min. to remove residual wash buffer. Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Note that residual ethanol from Buffer PE may inhibit subsequent enzymatic reaction.
10. Place the column in a clean 1.5 ml micro centrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris-HCl, pH 8.5) or water to the center of each spin column, let stand for 1 min, and centrifuge for 1 min.