

Plasmid 96 MiniPrep Kit

| Product | Catalog # | Purifications |
|---|-----------|---------------|
| Plasmid 96 MiniPrep Kit (2 plates) | 21914 | 192 |
| Plasmid 96 MiniPrep Kit (10 plates) | 49181 | 960 |
| Plasmid 96 MiniPrep Kit (No tips or growth blocks, 10 plates) | 65742 | 960 |

Description

Plasmid 96 MiniPrep Kit is a 96-well plate kit that utilizes an alkaline lysis protocol. Bacteria are grown in 1-1.3 ml volumes in a 96-well block, pelleted by centrifugation, and lysed in the same block. Samples are transferred to a 96-well filterplate and centrifuged into alcohol in a 96-well receiver plate. The pelleted DNA is washed with 70% ethanol and dried. Full processing time for 2 blocks is 45 minutes, (including time for pelleting bacteria). Processing time for 4 blocks (384 templates) is 60 minutes.

| Kit Components | 21914 | 49181 | 65742 |
|--|----------|-----------|-----------|
| TE Buffer | 25 ml | 110 ml | 110 ml |
| RNase Solution | 0.25 ml | 1.1 ml | 1.1 ml |
| Lysis Buffer | 20 ml | 100 ml | 100 ml |
| Neutralization Buffer | 20 ml | 100 ml | 100 ml |
| 96-Well Growth Blocks [2-ml capacity per well] | 2 blocks | 10 blocks | --none-- |
| 96-Well Filterplates [0.8-ml capacity per well] | 2 plates | 10 plates | 10 plates |
| 96-Well Receiver Plates [0.8-ml capacity per well] | 2 plates | 10 plates | 10 plates |
| Adhesive Plate Sealers | 1 pack | 1 pack | 1 pack |
| Large Orifice Tips | 192 tips | 960 tips | --none-- |

Equipment and Materials Required

1. Multichannel pipettor
2. Centrifuge capable of reaching 1500 x g
3. Carrier capable of holding a 96-well block, 5 cm in height*
4. Isopropanol
5. 70% ethanol

* Note: Not all centrifuge manufacturers that carry microplate carriers will meet this specification. Contact your centrifuge manufacturer for information. For additional information, contact the technical services department at Edge BioSystems, Inc. (800) 326-2685.

Storage and Stability Conditions

Store at room temperature. The kit is stable at this temperature for up to 12 months.

After addition of RNase Solution to the TE Buffer, store the mixture at 4°C.

Quality Control

Tested for performance in plasmid DNA isolation. Analyzed for purity by agarose gel electrophoresis and evaluated by restriction endonuclease digestion.

Warning: This product is intended for **research use only**. It is not to be used for diagnostic purposes in humans or animals.

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Recommended Protocol

1. **Fill the 96-Well Growth Block with 1–1.3 ml of sterile broth.**
2. **Inoculate wells with bacteria.**
3. **Culture 18–24 hours overnight at 37°C with vigorous shaking.**
 - An orbital shaker at 300-350 rpm is recommended.
4. **Centrifuge the 96-Well Growth Block in a microplate carrier at 1500 x g (see Notes) for at least 5 minutes.**
 - The clarity of the supernatant generally is a good indication of the adequacy of centrifugation. If the pellets can be seen clearly through the media, then they will be sufficiently compact to not dislodge while removing the media.
5. **Remove supernatant by decanting.**
 - Immediately, invert the block over a tray to remove the used broth, then tap the inverted block 2-3 times on a paper towel or adsorbent pad to remove remaining broth.
6. **Add 0.01 volumes RNase Solution to the TE Buffer. Mix well.**
 - For example, to 25 ml of TE Buffer, add 250 µl of RNase Solution.
 - After addition of RNase Solution to the TE Buffer, store the mixture at 4°C.
7. **Add 100 µl RNase/TE Buffer solution to sample.**
8. **Resuspend pellet by vortexing or by repeated pipetting.**
 - Complete dispersal of the pellet is critical to good yields and easy sample handling.
9. **Add 100 µl of Lysis Buffer.**
10. **Apply adhesive sealer evenly to top of block.**
11. **Mix by shaking laterally or partially inverting 10 times.**
 - Do not invert completely.
 - The lysate should be homogeneous and relatively clear after mixing. If not, continue mixing.
12. **Wait 5 minutes.**
13. **Remove adhesive sealer.**

14. **Add 100 µl of Neutralization Buffer.**
15. **Mix by pipetting 5 times vigorously or by vortexing.**
16. **Transfer sample to filterplate.**
 - Use of Large Orifice Tips is recommended.
17. **Wait 5 minutes.**
18. **Add 210 µl of isopropanol to the wells of the receiver plate.**
19. **Stack filterplate on top of receiver plate.**
 - Do not cover filterplate with adhesive sealers.
20. **Centrifuge contents of filterplate into receiver plate at 1500 x g for 15 minutes.**
21. **Decant supernatant.**
22. **Add 200 µl of 70% ethanol.**
23. **Decant alcohol and air dry.**

Notes

Conversion of RCF to RPM Calculation:

An accurate determination of the centrifugation speed is very important. The relative centrifugal force (RCF) specified in the protocol is converted to revolutions per minute (RPM) using the following formula:

$$RPM = 1000 \sqrt{\frac{RCF}{1.12 r}}$$

The radius, *r*, is equal to the distance in millimeters between the axis of rotation and the bottom of the plate.

To achieve RCF = 1500 x g:

$$RPM = 36,596 \sqrt{\frac{1}{r}}$$

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