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User's Guide

Global Genomics Partner

AccuPrep® Plasmid Mini Extraction Kit

Cat. No.: K-3030
Cat. No.: K-3030-1

BIONEER
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Safety Warnings and Precautions.

AccuPrep® Plasmid Extraction Kit is developed and sold for research purposes only. It is not recommended for human or animal diagnostic use, unless cleared for such purposes by the appropriate regulatory authorities in the country of use.

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information please consult the appropriate Material Safety Data Sheets (MSDS).

The Neutralization Buffer contains a chaotrophic salt and should be handled with care. Chaotrophic salts form highly reactive compounds when combined with bleach. Thus great care must be taken to properly dispose of this solution.

Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of Bioneer's quality management system from product development to production to quality assurance and supplier qualification meets or exceeds the world-class quality standards.

Trademarks

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AccuPrep® Plasmid Mini Extraction Kit
Technical Manual

I. Description

The *AccuPrep*® Plasmid Mini Extraction Kit was developed for the extraction of highly purified plasmid DNA from cultured bacterial cells within 20 min. The overall principle combines modified alkaline lysis method (Birnboim *et al*, 1979). Collected cells are re-suspended in Resuspension Buffer. Following the addition of Lysis Buffer and Neutralization Buffer to the lysate, the chromosomal DNA and cell debris will be forms an insoluble aggregate. The insoluble protein aggregate is removed following centrifugation and transfer the clear lysate to the DNA binding filter tube. The cleared lysate contains a chaotropic salt originating from Neutralization Buffer which helps the binding of the plasmid DNA on the membrane surface. The DNA binding filter which is packed with silica based membrane has enough surface area to bind up to 20 μg of plasmid DNA. Any salts and precipitates are eliminated by addition of the Washing Buffer. Finally, highly purified plasmid DNA can be eluted with Elution Buffer or Nuclease free autoclaved distilled water(not provided).

II. Kit Components

Cat. No	K-3030	K-3030-1
Buffer ①	60 ml	15 ml
Buffer ②	60 ml	15 ml
Buffer ③	80 ml	20 ml
Buffer D	75 ml	18 ml
Buffer ④	2×16 ml	2×4 ml
Buffer ⑤	24 ml	6 ml
RNase A powder	6 mg	1.5 mg
DNA binding column tube	200 ea	50 ea
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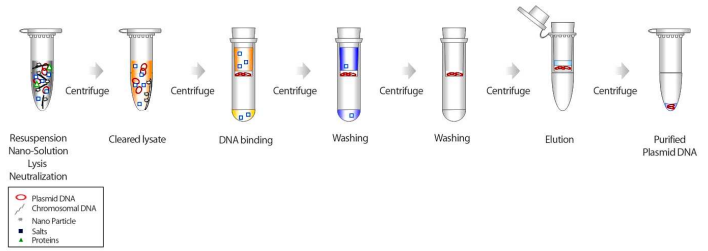
※ Buffer ③ and Buffer D contains chaotropic salt and should be handled with care. Chaotrophic salts can makes highly reactive compounds when mixed with disinfecting agent such as bleach.

※ All buffers and DNA binding columns can be stored under room temp. But, Buffer ① must stored at 4□ after addition of RNase A powder.

III. Procedure



IV. Before You Begin

Before you start your prep, please check the followings.

1. Chemicals
- Did you add RNase A powder to Buffer ① and completely dissolve it?
- Did you add absolute EtOH to Buffer D as described below?
- Did you add absolute EtOH to Buffer ④ as described below?

Cat. No.	K-3030	K-3030-1
Buffer D	75 ml	18 ml
Absolute EtOH	45 ml	10.8 ml
Total	120 ml	28.8 ml

Cat. No.	K-3111	K-3112
Buffer ④	16 ml	4 ml
Absolute EtOH	64 ml	16 ml
Total	80 ml	20 ml

2. Equipments
- High speed table top centrifuge (capable of ≥13,000 rpm, 4□)

Are you ready to prep your Plasmid DNA?

V. Experimental Protocol for Mini Prep.

■ ***E.coli*** **cell preparation**

1. **Pick up a single colony from fresh cultured LB(Luria-Bertani) agar plate(contains antibiotics) or your established media and inoculate the cell into the 1-5 ml of fresh LB liquid media (contains antibiotics) or your established media at 37℃ with shaking for 12-16 hr.**
   Do not over-growth your *E.coli* cell. It will decrease the productivity because of the cell death and inefficient lysis.
   For high copy number plasmid DNA : 1~5ml of *E.coli* cells
   For low copy number plasmid DNA : 1~10ml of *E.coli* cells

2. **Collect the *E.coli* cells by centrifugation at >8,000 rpm for 2 min. or >3,000 rpm for 5 min. And completely remove of the media by pipetting.**

■ **Cleared lysate preparation**

1. **Add 250 µl of Buffer ① to the collected cells and completely resuspend by vortexing or pipetting.**
   Complete resuspension will make high lysis efficiency.

2. **Add 250 µl of Buffer ② and mix by inverting the tube 3-4 times gently.**
   Avoid vortex! Vortexing may cause shearing of genomic DNA. It is important to invert gently.

3. **Add 350 µl of Buffer ③ and immediately mix by inverting the tube 3-4 times, gently.**
   Genomic DNA and cell debris will form an insoluble complex. Again, be cautions not to shear genomic DNA.

4. **Centrifugation the tube at 13,000 rpm, 4□ for 10 min. (>16,600 ×g) in a micro-centrifuge.**
   After centrifugation, white protein aggregate will appear at the bottom of the tube. If your centrifuge is not enough to get a cleared lysate, please centrifuge again.

■ **Plasmid DNA Purification**

5. **Transfer the cleared lysate to the DNA binding column tube and centrifuge at 13,000 rpm for 1 min. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**

6. **(Optional) Add 500 µl of Buffer D and wait for 5 min. and centrifuge at 13,000 rpm for 1 min. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**
   This step is required if you are using an *endA*$^+$ strains which has a high endonuclease activity. BL21, CJ236, HB101, JM83, JM 101, JM110, LE392, NM series strains, PR series strains, Q358, PR1, TB1, TG1, Y10 series strains, BMH71-18 and ES1301 are *end*A$^+$ strains, thus they produce highly active endonucleases that can degrade plasmids. Denaturation step is not required for the **DH5α**, **XL1-Blue**, BJ5183, DH1, DH20, DH21, JM103, JM105, JM106, JM107, JM108, JM109, MM294, SK1590, SK1592, SK2267, SRB and XLO strains.

7. **Add 700 µl of Buffer ④ to the DNA binding column tube and centrifuge at 13,000 rpm for 1 min. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**
   This removes salts and soluble debris. The amount of plasmid washed away in 80 % ethanol is negligible.

8. **Dry by additional centrifugation at 13,000 rpm for 1 min. to remove the residual ethanol.**

9. **Transfer the DNA binding filter column to the new 1.5 ml microcentrifuge tube (not provided).**

10. **Add 50-100 µl of Buffer ⑤ to the DNA binding filter column, and wait for at least 1 min. for elution.**
    If you want to get a more concentrated solution of plasmid, a smaller volume is appropriate, but total yield may be reduced. If the plasmid is low copy or larger than 10 kb, the yield of plasmid may not be sufficient. Pre-warmed (about 60 □) elution buffer will improve efficiency of elution.

11. **Elute the plasmid DNA by centrifugation at 13,000 rpm for 1 min.**
    If you want more quantity, elute the sample twice and use after concentrating process.

VI. Troubleshooting

1. Low yield of plasmid

1) Did you harvest a sufficient amount of cells ? The yield is dependent on the host strain type and an overload of cells may decrease the yield.

2) Did you completely resuspend the cells with Buffer ① ? Incomplete resuspension decreases the efficiency of lysis.

3) Is there precipitated salt in the Buffer ③ ? Vortex or shake well to re-dissolve the precipitant. An improper concentration of the chaotrophic agent will decrease the yield. If it does not re-dissolve easily, warm it to 60 ℃.

4) Has it been over 6 months since you added RNase A powder? Low concentration of RNase A can result in a low yield of plasmid. After about 6 months, add more RNase A, up to 100 **μg/μl.**

2. Contamination of chromosomal DNA (The appearance of unexpected bands following gel electrophoration).

During neutralization step, samples should not be vortex or shaken vigorously. Also, the period of lysis should not be longer than 5 min. Both can shear the chromosomal DNA. Handle the lysate gently!

3. Sample floats upon loading in agarose gel.

Sample contains alcohol. Floating is caused by leftover ethanol. You must always dry the column completely by centrifugation and make sure that no droplet is hanging from the tip of the binding column.

4. Too many background bands appear in sequencing analysis.

Did you check the endonuclease activity of your strain of host *E. coli*? HB101, JM series and normal wild-type hosts that have high endonuclease activity interrupt the sequencing reaction by degrading the plasmid. We recommend using the *EndA*$^-$ strain instead of *EndA*$^+$ strain.

5. Sample contains RNA.

1) RNase activity is weakened. If it has been over 6 months since adding the RNase A powder to the Resuspension, the RNase A may not work properly. Add more RNase A powder, up to 100 μg/μl.

VII. References

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