USER'S GUIDE

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AccuPrep®
Plasmid Mini Extraction Kit

REF K-3030
K-3030-1

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AccuPrep® Plasmid Mini Extraction Kit

Kit for the extraction of Plasmid from bacterial culture

User's Guide

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Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® Plasmid Mini Extraction Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety warning and Precaution

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 Sheet (MSDS).

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.

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Contents

1. Description

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 formed 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).

Advantages:

- ✓ Plasmid DNA can be extracted quickly and more conveniently.
- ✓ Contaminants, such as proteins and nucleases, which may interfere with PCR reactions, are completely removed, improving the efficiency and reproducibility of PCR.
- ✓ Damage to Plasmid DNA is minimized by avoiding precipitation and use of organic solvents.
- ✓ The isolated Plasmid DNA is ready for use in various applications.

Applications

- ✓ Gene Cloning
- ✓ PCR
- ✓ Real time PCR

2. Kit components

This kit will maintain performance for at least two years under standard storage conditions.

Reagents	K-3030	K-3030-1
RNase A powder, lyophilized	6 mg	1.5 mg
One vial with lyophilized RNase A is included. Before first use, Add the provided RNase A to PA1 Buffer.		
PA1 Buffer	60 ml	15 ml
Mix PA1 Buffer thoroughly by shaking before use. PA1 Buffer is stable for 2 years when stored at room temperature (RT, 15 – 35℃). But PA1 Buffer must be stored at 4℃ after addition of RNase A powder.		
P2 Buffer	60 ml	15 ml
P2 Buffer is stable for 2 years when stored at RT.		
PA3 Buffer	80 ml	20 ml
PA3 Buffer is stable for 2 years when stored at RT.		
PB Buffer	75 ml	20 ml
PB Buffer is supplied in a concentrated form. Before first use, add appropriate volume of absolute ethanol to PB Buffer as printed on the product label. PB Buffer is stable for 2 years when stored at RT.		
BST Solution	40 ml	10 ml
Store at room temperature.		
W2 Buffer	2 x 80 ml	40 ml
W2 Buffer is stable for 2 years when stored at RT.		
EA Buffer	25 ml	15 ml
10 mM Tris-HCl (pH 8.0). Store at RT.		
Columns and tubes		
Binding column tubes	200 ea	50 ea
Collection tubes (for filtration)	200 ea	50 ea

Additional required materials

- ✓ Table-top microcentrifuge, 16,000 xg (>13,000 rpm)
- ✓ Vortex mixer
- ✓ 1.5 ml tube
- ✓ Absolute ethanol

3. Before you begin

Before proceeding, please check the following:

- **Did you add RNase A powder to PA1 Buffer and completely dissolve it?**
- **Did you add the correct amount of absolute ethanol to solution PB Buffer?**

Cat. No.	K-3030	K-3030-1
PB Buffer	75 ml	20 ml
Absolute ethanol	45 ml	12 ml
Total	120 ml	32 ml

☞ PA3 Buffer and PB Buffer contain chaotropic salt. You should take the appropriate laboratory safety precautions and wear gloves when handling.

☞ The g-force can be calculated as follows:

RCF = 1.12 x r x $(rpm/1,000)^2$

Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

4. Experimental protocol

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 3,000×g (>8,000 rpm) for 2 min. or 600×g (>3,000 rpm) for 5 min. And completely remove of the media by pipetting.**

3) **Add 250 µl of PA1 Buffer to the collected cells and completely resuspend by vortexing or pipetting.**

   Complete resuspension will make high lysis efficiency.

4) **Add 250 µl of P2 Buffer 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.

5) **Add 350 µl of PA3 Buffer 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.

6) **Centrifugation the tube at 16,000×g (>13,000 rpm), 4℃ for 10 min. in a micro-centrifuge.**

   After centrifugation, white protein aggregate and Nano-particle complex will appear at the bottom of the tube.
   If your centrifuge is not enough to get a cleared lysate, please centrifuge again.

7) **Add 100 µl of BST Solution to the binding column tube (fit in a collection tube) and centrifuge for 30 sec at 13,000 rpm.**

8) **Discard the solution from the collection tube and reuse the collection tube.**

9) **Transfer the cleared lysate to the Binding column (fit in a collection tube) and centrifuge at 13,000 rpm for 1 min. Pour off the flow-through and re-assemble the Binding column with collection tube.**

10) **(Optional) Add 500 µl of PB Buffer and wait for 5 min. and centrifuge at 16,000×g (> 13,000 rpm) for 1 min. Pour off the flow-through and re-assemble the DNA binding column with 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 endA+ 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.

11) **Add 700 µl of W2 Buffer to the DNA binding column tube and centrifuge at 16,000×g (>13,000 rpm) for 1 min. Pour off the flow-through and re-assemble the DNA binding 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.

12) **Dry the DNA binding filter by additional centrifuge at 16,000×g (>13,000 rpm), room temperature for 1 min to remove the residual ethanol.**

If the liquid has not completely passed the column following centrifugation, then centrifuge again until the liquid completely passes through.

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

14) **Add 50 – 100 µl of EA Buffer 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 ℃) EA Buffer will improve efficiency of elution.

15) **Elute the plasmid DNA by centrifugation at 16,000×g (>13,000 rpm) for 1 min.**

If you want more quantity, elute the sample twice and use after concentrating process.

5. Troubleshooting

1) There is a low yield of DNA or low levels of purity of Plasmid.

① **Buffers or other reagents may have been exposed to conditions that reduce their effectiveness.**
Ensure that the reagents were stored at room temperature (15 – 25°C) at all times upon arrival and all reagent bottles were closed tightly after use to preserve pH, stability, and to avoid contamination.

② **The cells may not have been completely resusended with PA1 Buffer.**
Incomplete resuspension decreases the efficiency of lysis.

③ **There is precipitated salt in P2 Buffer and PA3 Buffer.**
Vortex or shake well to re-dissolve the precipitant. An improper concentration of the buffer will decrease the yield. If it does not re-dissolve easily, warm it to 60 ℃.

④ **You may not have used the optimal reagent for eluting Plasmid.**
An alkaline pH is required for optimal elution. Use the EA Buffer included in the kit.

⑤ **There is a low recovery of Plasmid following elution.**
You may not have used the optimal reagent for eluting the DNA. An alkaline pH is required for optimal elution. Next time, do not use water to elute DNA. Use EA Buffer included in the kit.

2) There is contamination of chromosomal DNA (Appearance of unexpected bands following gel electrophoresis).

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.

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 ng/μl

6. Supplementary material

Typical Results

The yield and purity of Plasmid DNA varies depending on the sample. The table below shows experimental results.

Sample	Amount of sample	Yield (μg)
E.coli	1 ml ~ 5 ml	Up to 20 μg

7. Explanation of symbol

	Catalog Number		Contains sufficient for (n) tests
	USE BY		Temperature Limitation
	Batch code		Caution, consult accompanying documents
	Manufacturer		Caution, Potential Biohazard
	DO NOT REUSE		Consult Instruction For Use

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