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User's Guide

Global Genomics Partner

AccuPrep® Plasmid Extraction Kit

for 96 well vacuum block

Cat. No.: K-3030-2

BIONEER
bioneer corporation

Safety Warnings and Precautions.

For research use only. It is not recommended to diagnose disease in humans or animals. DO NOT use to humans or animals.

Wear gloves when you are handling irritant or harmful reagents.

Warranty and Liability

All BIONEER products are tested under extensive Quality Control procedures. BIONEER guarantees the quality under the warranty period. Any problems should be reported immediately. Liability is conditional upon the customer providing full details of the problem to BIONEER. Once the problem occurs, customer must report to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

QC Testing

Each lot of BIONEER's product is carefully tested in the quality control team.

Trademarks

***AccuPrep*®** ,96 well vacuum block™ and *HT-MegaGrow*™ are trademark of Bioneer Corporation.

Contents

AccuPrep® Plasmid Extraction Kit
For 96well Vacuum Block
Technical Manual

I. Description

This product is designed for the rapid extraction of up to 12μg of different kinds of high-purity plasmid DNA from bacterial cultures such as *E. coli*, by using the standard 96-well type plate. After 12~16hrs, the concentrated *E. coli* liquid culture is lysed using the Modified Alkaline Lysis method (Birnboim, H.C and J. Doly. 1979). And RB buffer lowers the pH value of the solution, allowing chromosomal DNA and cell debris to be aggregated.

In addition, the RB buffer contains chaotropic salts that enhance the binding of DNA with the filter surface and activate the binding. After vacuum filtering, the contaminants, such as salts and other proteins, are filtered through by using trapping plate and only the plasmid DNA binds to the binding plate that contains filter fibers. The binding plate is washed using WB buffer after the process, and then dried completely. High-purity plasmid is obtained by dissolving the plasmid DNA in EL Buffer.

II. Kit Components

This product has been designed for extraction of plasmids from 192(96X2) different samples, and will maintain its activity for at least a year.

***AccuPrep*® Plasmid Extraction Kit for 96 well vacuum block**

Cat. No.	K-3030-2
RS Buffer	**30 mL X 2**
Add 1 RNase A powder (3.0mg) to each bottle. Wash the bottles several times using RS Buffer to remove RNase A powder. Store at 4°C after the addition of RNase A. The added RNase A will retain its activity for up to 6 months. After longer periods of storage, RNase A may not eliminate RNA effectively. In the case of presence of RNA in the purified plasmid, add more RNase A powder, up to 100 μg/mL. RNase A of that concentration will work very well.	
RNase A Powder	**3.0 mg X 2**
Add this powder to RS Buffer. Wash several times with RS Buffer and remove.	
BL Buffer	**60 mL**
Store at room temperature (20~25°C). If salts are precipitated, they should re-dissolved before use.	
RB Buffer	**60 mL**
Store at room temperature (20~25°C). If salts are precipitated, they should re-dissolved before use.	
DE Buffer	**75 mL**
Add 60 mL of absolute ethanol before use. Store at room temperature (20~25°C).	
WB Buffer	**20 mL X 2**
Add 200 mL of 96~100% ethanol to each bottle.	
EL Buffer(10 mM Tris-HCl pH 8.5)	**60 mL X1**
Store at room temperature.	

Plate and Cover	
96 well trapping plates	2 ea
96 well binding plates for plasmid	2 ea
96 well dome plates	2 ea
96 RV Plate	2 ea
96 well sealing tapes	4 ea

III. Additional reagents & instruments

HT-MegaGrow™(Cat. No., A-4080)
96 well vacuum block™ (Cat. No., A-9030)
Multichannel Pipette
Ethanol (96-100%)

IV. Before Starting

1. **Before you proceed, check 'VIII. Remark' first.**
2. **Adjust the degree of the vacuum on Vacuum block**
   By putting tray in vacuum block and equipping with a new binding plate, you can measure the degree of the vacuum. Adjust the degree of the vacuum to **-60 ~ -150mmHg.** If it is too high, the solution may be splashed, and if it is too low, the filtering term may be longer. The method of adjusting the degree of the vacuum is as follows: after putting a tray on the bottom of the vacuum block, you need put a new or used binding plate on it. Add about 100 μL of distilled water, and measure by producing vacuum. In the case of using new plates, it should be completely dried before use.
3. **Did you add RNase A powder to RS Buffer?**
   Refer to 'Kit Components' part.
4. **Did you add adequate amount of absolute ethanol to DE Buffer?**
   Add 60 mL of absolute ethanol.
5. **Did you add adequate amount of 96~100% ethanol to WB Buffer?**
   Add 200 mL of 96~100% ethanol to the offered 20 mL of WB Buffer.
6. **Is BL Buffer or RB Buffer precipitated?**
   If the salts are precipitated, it should be dissolved completely before use. If it does not dissolved well, it may

be heated up to 50~60°C.

7. **Management of unused wells**
   It is recommended to fill all wells with samples, however, if part of wells are used, the rest of wells should be blocked using tape etc. In this case, the offered 96 well sealing tape is not to be used; it has another purpose.

V. EXPERIMENTAL PROTOCOL

1. **Harvest *E. coli* cells.**
2. **Add 200 μL of re-suspension RS Buffer and then transfer to the trapping plate.**
3. **Remove the solution from the trapping plate, and place the Binding plate on the trapping plate.**
4. **Add 200 μL of BL Buffer to each trapping plate, and seal them with Sealing tape, and mix gently by inverting. All reactions should be done within 5 minutes.**
5. **Remove the Tape, and eliminate the solution remaining on the top surface of trapping plate.**
6. **Add 200 μL of RB Buffer to each Trapping plate, and seal them with Sealing tape, and mix gently by inverting.**
7. **After 5 minutes, the solution in trapping plate is eluted in Binding plate by vacuum filtering.**
8. **The filtered solution in Binding plate is extracted by vacuum filtering.**
9. **(Options) In the case of *end*A+ strain (Refer to Remarks.), add 500 μL of DE Buffer, and after about 5minutes, perform vacuum filtering.**

10. **Add 1 mL of WB Buffer to Binding plate. It should be decompressed and filtered.**

    Pour out the solution obtained in Tray. Binding plate is equipped at the top, and under the vacuum condition, it should be dried for 20 minutes.
11. **Dry the bottom of Binding plate.**
12. **96 well RV plate is equipped with a vacuum block at the bottom, and with Binding plate at the top. Add 100 μL of EL Buffer to each well. React for 5-10 minutes.**
13. **The purified plasmid solution may be obtained by the decompression, and filtration processes.**

VI. PROTOCOL IN DETAIL

Add adequate amount *E. coli* cells to 96 well dome plate, and centrifuge.
Centrifuge for about 15 min. at 100g(~2,000rpm). **Centrifuge samples until the media becomes be clear.**

1. **Invert the plate promptly, and tap it gently so that the components of media are removed.**
   Strong tapping may result in the drop of cell pellet. Tap gently and wipe the entrance with paper scraps.
2. **Add 200 μL of RS Buffer, fully re-suspend the cells, then transfer them to Trapping plate.**
   96 well vortexer may be used during the cell re-suspension, or the cells may be transferred to Trapping plate using multi-channel pipettes. Incomplete re-suspension of cell pellets may lower the yield of plasmid.
3. **Binding plate is placed at the bottom of Trapping plate that contains transferred solution.**

This prevents exposure to the liquid leaked from trapping plate.

4. **Add 200 μL of BL Buffer to each Trapping plate, and seal it using Sealing tape, with the piled Binding plate, gently invert 4~6 times and mix. Time for all reactions should proceed within 5 minutes.**
   Do not mix the solution vigorously. Vigorous mixing may not eliminate Genomic DNA. After sealing with tape, the plate must be scrubbed with soft roller or paper scraps.

5. **Remove the tape, and gently tap the plate with paper scraps so that solution remaining on the surface of Trapping plate is removed.**

6. **Add 200 μL of RB buffer to Trapping plate, after sealing with new Sealing tape, gently invert and mix the trapping plate placed with Binding plate 4~6 times.**
   Do not mix the solution vigorously. Vigorous mixing may not eliminate Genomic DNA. After sealing with tape, the plate must be scrubbed with soft roller or paper scraps.

7. **Equip the piled Binding plate with 96well vacuum block at the bottom, and put Trapping plate on the top of the block. Wait for about 5 minutes.**
   Since the solution may leak from the bottom of trapping plate, you must be cautious of cross-contamination while separating two plates. If necessary, gently wipe off the bottom with paper scraps. After 5 minutes, the solution remaining on the sealing tape may go down.

8. **Remove the Sealing tape, and filter by vacuum.**
   It is not necessary to remove the solution on the surface. In general, the filtration will be done in 7 minutes, however, the filtration may last about 20~30 minutes if too many samples are used**. The filtration time depends on the amount of cells(Refer to "Problem Solving").**

   Discard Trapping plate, equip tray at the bottom and place the binding plate (previously equipped at the

bottom) on the top to filter by vacuum.
While discarding the trapping plate, you must be cautious that the droplets hanging on the bottom may cause cross-contamination.

9. **(Options) Add 500 μL of DE Buffer to each well, wait for about 5 minutes, and filter by vacuum.**
   This step requires *end*A+ strain. Other strains cannot be used in this step(Refer to 'Remarks'.).

10. **Add 1 mL of WB Buffer to Binding plate and filter completely by vacuum.**

11. **Repeat process (12) mentioned above One more time.**
    Washing step mentioned above is recommended to be proceeded through two steps; using 1.2 mL,, and 0.8 mL. This is for complete elimination of the solution remaining on the top wall of binding plate. Using 1 mL, wash twice. The solution not removed during the washing step should not leak to the below filter**.**

12. **Pour out the solution obtained in tray, and equip the tray at the bottom. Put Binding plate on the top and then dry for 20 minutes under vacuum condition.**
    Adjust the vacuum to the maximum, which means, you must make sure that the vacuum regulator is running with maximum decompression. Excessive drying may result in low elution efficiency.

13. **Tap and strongly shake off binding plate until the moisture is dried. Tap and wipe the nozzle tip again with paper scraps.**

14. **Place 96 well RV plate at the bottom of vacuum block, and equip with Binding plate on the top of it, then add 100 μL of Elution buffer. All reactions proceed for 5-10 minutes.**
    The middle of filter should get wet, so the yield will be high.

15. **Obtain the purified plasmid solution by vacuum filtering.**

VII. Trouble Shooting

1. Low yield of plasmid

Did you use a sufficient amount of culture solution? If not, you should use sufficient culture solution. In the case of using *HT-MegaGrow*™ (Cat. No., A-4080),it is recommended to use longer culture. However, if cells are cultured too long (over 24hrs), the yield of cells will decrease with the number of not good cells increasing. On the other hand, if too much liquid culture solution is used compared to that elucidated in the protocol, the low efficiency of lysis will result in low cell yield.

1) Are the salts in BL or RB Buffer precipitated? Salt precipitation in each buffer decreases the efficiency of the solution significantly.
2) Has it been over 6 months since you added RNase A to RS Buffer? If so, add more RNase A, up to 100μg/mL. Since RNA is also absorbed to the surface of filter, if RNA is not completely eliminated, adsorption of DNA to the surface of filter will be interfered due to the competition effect of RNA and DNA adsorption. In this case, RNA may remain in the purified plasmid solution.
3) The yield of cells depends on plasmid, host and insert. In this case, process centrifugation before use.

2. Incomplete filtering through by trapping filter

Too many cells were harvested. We recommend to use appropriate amount of cells. If it isn't filtered through by vacuum filtering for more than 20~30 minutes, sufficient plasmid will be obtained.

3. Sample contains RNA

1) Did you add RNase A powder to RS Buffer?
2) Too many cells were harvested. Adequate amount of culture solution should be used. In the case of using *HT-MegaGrow*™(Cat. No., A-4080), longer culture time is recommended.
3) The activity of RNase A may be weakened. If it has been over 6 months since you added RNase A to RS Buffer, more RNase A should be added, up to 100 μg/mL.

4. Incompletely digested by restriction endonuclease

When too much ethanol remained in the filtrate, you must dry for longer time. If the problem still occurs, completely dry the finally obtained plasmid, and then re-dissolved them in distilled water before use.

5. Contamination of chromosomal DNA

1) Samples should not be vortexed or shaken vigorously during the Lysis process (after the addition of BL Buffer and RB Buffer). Solution should be mixed gently.
2) Cells were cultured for too long time. If cells are cultured for longer than recommended E.coli cell culture time, the amount of dead cells increases. Therefore, the cells naturally lysed in the media, fragments of DNA, may be leaked into the media.

6. Sample floats upon loading in agarose gel

If the sample contains too much alcohol, you must dry the column completely. If another problem still occurs, you must dry the obtained plasmid DNA solution completely, and then re-dissolved in distilled water before use.

7. Too many background bands appear in the sequencing analysis

Did you check the endonuclease activity of your *E. coli*

strain? Part of *end*A+ strain has high endonuclease activity, so that the sequencing reaction may be interrupted. (Refer to 'VIII. Remark' part.)

1) 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 50 ℃.

2) 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.

8. Contamination of chromosomal DNA (The appearance of unwanted bands following gel analysis).

During step 3, samples should not be vortexed or shaken vigorously. Also, the lysis time should not exceed 5 min. Both can shear the chromosomal DNA. Handle the lysate gently!

9. Sample floats upon loading in agarose gel

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

10. 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. For these types, step 7, denaturation, is required.

VIII. Remarks

E. coli strain

Use the host with low endonuclease activity, which does not produce various carbon hydrates. Using strains such as XL1-Blue and DH5α doesn't cause these problems.
When using *end*A+ strain (BL21(DE3), CJ236, HB101, JM83, JM101, LE392, MC1061, all NM series, P2392, and all PR series, Q358, RR1, TB1, TG1, all Y10 series), the quality of final amount of obtained plasmid may decrease. In this case, each sequential enzymatic reaction or sequencing reaction may proceed incorrectly. In addition, the yield of plasmid extracted from such hosts is low, therefore, it is recommended not to use them except for special cases.

Culture and Harvest

Culture

1. **Using *HT-MegaGrow*™(Cat. No., A-4080)**
   Add 300-700μl of sterilized TB media to 96well dome plate. Adding too many cells may overflow the liquid culture solution. Add adequate antibody and incubate the cells. Cell culture process should be done at 700 rpm, since more rotations will provide sufficient oxygen, and decrease cell density. However, too high rpm will cause damage to the machine. (Do not set up over 700rpm.) Oxygen is injected for 5minutes between the time intervals of 20 minutes. The pressure of oxygen injection should be 2~3 kgf/cm^2. Do not set up the input pressure higher than 7 kgf/cm^2. The cells will be cultured for 12~24 hrs.

2. **Not using *HT-MegaGrow*™(Cat. No., A-4080)**

Both LB and TB may be used (the amount of LB should be 1~4mL (O.D. (600nm) > 1), and TB, about 0.4mL ~1.0mL.) Add adequate antibody to the media and incubate the cells. The cells will be cultured for about 12~24 hrs.

Harvest

If the cells are cultured in 96 well dome plate, the cells should be centrifuged by the machine that can hold 96 well plate. Centrifuge for approximate 5~10 minutes at 1,000g (~2,000rpm). If the speed is over 1,000g, the plate may be damaged. Unclear supernatant should be centrifuged for longer time. The media components may be removed with aspirator or by rapidly inverting and gently tapping the plate. Be cautious that strongly tapping may result in the drop of cell pellet. If the cells are cultured with other methods, transfer the liquid culture solution into 96well dome plate. (Can add up to 1mL to each well) The cells are concentrated using the same method. If the cells are not sufficient, add liquid culture solution again concentrate the mixture several times.

Lysis

Use the Sealing Tape. Add BL and RB buffer before use. Straighten the tape before sealing (confirm folds), and rub it strongly with soft roller or a bundle of paper scraps. Cross contamination may happen when sealed not properly.

Drying

The elution yield decreases if ethanol remains on the filter fiber. Products containing ethanol can't normally perform enzymatic reaction. Therefore, the plate should be completely dried. The

time should be adjusted according to the humidity in the air, however, in general, the plate should be dried for 20 minutes under vacuum condition(200~600 mmHg). Excessive drying will also lower the yield.

Elution

By adjusting the amount of EL Buffer, the final concentration of plasmid may be controlled. The relation between the amount of EL Buffer and recovery is showed in the following table (Table-1). If the concentration of product is low, completely dry and then add adequate distilled water before use.

Table-1. Recovery Yield according to Elution volume

Elution volume (μL)	70	80	90	100	150	200
Capable recovery volume (μL)	20	30	40	50	100	150
Concentration (ng/μL)	200	180	160	150	100	75
Amount of recovery (μg)	4	5.4	6.4	7.5	10	11

Distilled water may be used for EL Buffer. However, in this case, using pH adjusted distilled water is recommended. In general, when the pH value exceeds 7.5, the elution effect of is strong.

IX. REFERENCE

1. Birnboim, H.C and J. Doly. 1979. *Nucleic Acids Res*., **7,** 1513
2. Boom, R. *et al.* (1990), *J Clin. Microbiol*., **28**, 495-503
3. Carter, M.J. and Milton, I.D. (1993) *Nucleic Acids Res*., **21**, 1044
4. Melzak, K. A. *et al*. (1996), *J Colloid and Interface Sci*., **181**, 635-644
5. Taylor, R. G., Walker, D. C. and McInnes, R. R. (1993) *Nucleic Acids Res*., **21**, 1577-1678
6. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning 2nd ed*.

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