ISO 9001
Certified

Ver. 1.0

User's Guide

Global Genomics Partner

AccuPrep® 96 well Genomic DNA Extraction Kit for 96 well vacuum manifold

Cat. No. K-3032-2

BIONEER
bioneer corporation

Safety Warnings and Precautions

AccuPrep® Genomic DNA 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).

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

AccuPrep® is registered trademark of Bioneer Corporation in Korea.

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I. Kit Components

Product	Qty.
Binding Buffer (B)	50 ml X 1 ea
Washing Buffer 1 (W1)	80 ml X 1 ea
Washing Buffer 2 (W2)	50 ml X 1 ea
Elution Buffer (E)	50 ml X 1 ea
Proteinase K powder	25 mg X 4 ea
96 well binding plate	2 ea
96 well dome plate	2 ea
96 well RV plate	2 ea
Sealing film	20 ea
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※ Dissolve the 25 mg of lyophilized Proteinase K powder with 1.25 ml of nuclease free water. And after dissolving the proteinase K, please store under -20℃.

※ Add 60 ml of absolute ethanol to 80 ml of the Washing buffer 1 (W1), before use.

※ Add 200 ml of absolute ethanol to 50 ml of the Washing buffer 2 (W2), before use.

II. Additional reagents & instruments

- BioVac™ 96 vacuum manifold (Cat. No., A-9030)
- Multi-channel Pipette
- Absolute ethanol (96-100%)

III. Before Starting

1. **Adjust the degree of the vacuum on Vacuum block**
   AccuPrep® 96 well Genomic DNA Extraction Kit needs at least ~**300mmHg** for the successful filtration. If it is too high, the solution may be splashed, and if it is too low, the filtering term may be longer.
2. **Did you add adequate amount of absolute ethanol to Washing buffer 1 (W1)?**
   Add 60 ml of absolute ethanol.
3. **Did you add adequate amount of absolute ethanol to Washing buffer 2 (W2)?**
   Add 200 mL of absolute ethanol.
4. **Is Binding Buffer precipitated?**
   If the salts are precipitated, it should be dissolved completely before use. If it does not dissolved well, it may
5. **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.

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IV. Storage

AccuPrep® 96 well Genomic DNA Extraction Kit provides whole reagents for the genomic DNA extraction from various sample sources, e. g. whole blood, buffy coat, lymphocytes, plasma, serum, body fluids and cultured cells using vacuum manifold. All buffers can be stored dry at room temperature (15℃-25℃) for up to 2 years without open.

AccuPrep® 96 well Genomic DNA Extraction Kit provides lyophilized Proteinase K for the convenient use.
Lyophilized Proteinae K powder can be stored at room temperature (15℃-25℃) up to 2 years without any reduced activity. And dissolved enzymes must be stored at -20℃ for longer storage.

V. Introduction

AccuPrep® 96 Genomic DNA Extraction Kit allows fast and easy preparation of genomic DNA from 96 different samples, such as whole blood, lymphocyte and cell media.

It has high concentrated chaotropic salt bound to fixed glass fiber in column, and protein and other contaminants are eliminated through washing step. DNA is isolated and purified by elution step.

The kit is safe and convenient because organic solvent or ethanol precipitation is not needed in all steps. Samples possibly to be applied for DNA extraction by the kit are citrate, whole blood, buffy coat, lymphocytes, plasma, serum, body fluids or cultured cell which was treated with EDTA.

VI. Genomic DNA Extraction

1. **Add 20 µl of Proteinase K solution into each well of 96 well dome plate.**

2. **Add 200 µl of Whole blood sample into each well.**
   Complete resuspension will make high lysis efficiency.

3. **Add 200 µl of Binding buffer (B) and covered with sealing film. And completely resuspend by pipetting.**
   Complete resuspension will make high lysis efficiency.

4. **Incubate the 96 well dome plate at 60℃ for 10 min.**

5. **Remove the sealing film without cross-contamination, carefully and add 100ul of isopropanol. And mix the sample gently.**

6. **Remove the sealing film without cross-contamination and transfer the sample into the 96 well binding plate.**

7. **Place the Waste tray at the 96 well vacuum manifold and load the plate spacer. And place the 96 well binding plate at the top of the plate spacer as below (Fig. 1)**

8. **Remove the sealing film without cross-contamination and turn on the vacuum pump until the filtrate completely passed the trapping plate.**
   Unused wells must be sealed with sealing film, completely. After filtration, white protein aggregate will appear at the bottom of the trapping plate. If your filtration is not enough to get a cleared lysate, please increase the filtration time.

9. **Add 500 µl of Washing buffer 1 (W1) into the 96 well binding plate and turn on the vacuum pump until the filtrate completely passed the 96 well binding plate.**
   Unused wells must be sealed with sealing film, completely.

10. **Add 500 µl of Washing buffer 2 (W2) into the 96 well binding plate and turn on the vacuum pump until the filtrate completely passed the binding plate.**
    This removes salts and soluble debris. Unused wells must be sealed with sealing film, completely.

11. **Repeat step 10 for the complete washing.**

12. **Dry the 96 well binding plate to remove the residual ethanol at 60℃ dry oven.**

13. **Remove the Waste tray from the 96 well vacuum manifold and place the 96 well RV plate, plate spacer. And place the 96 well binding plate at the top of the plate spacer as below (Fig. 2).**

14. **Add 100-200 µl of Elution buffer (E) to center of the 96 well binding plate and wait for at least 5-10 min. for elution.**
    If you want to get a more concentrated solution of genomic DNA, a smaller volume is appropriate, but total yield may be reduced. Pre-warmed (about 60 ℃) elution buffer will improve efficiency of elution.

15. **Turn on the vacuum pump and elute the genomic DNA.**
    Unused wells must be sealed with sealing film, completely.

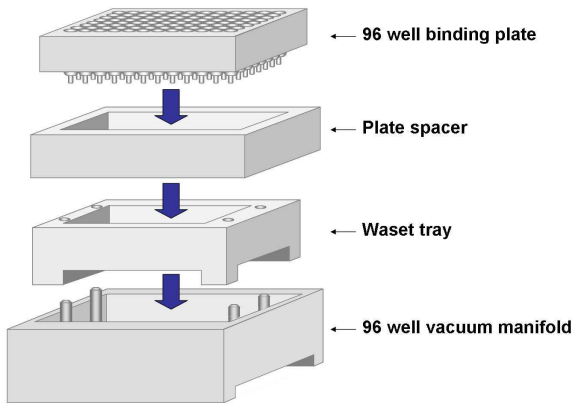

Fig. 1

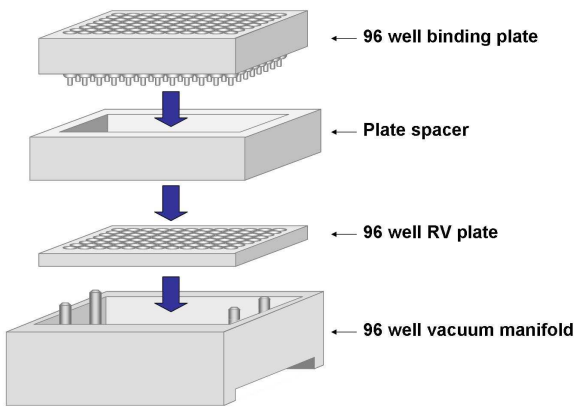

Fig. 2

VII. Troubleshooting

1. Low yield of recovery or low DNA preparation

1) Store the kit at 15 ~ 25 ℃

2) Especially, store the solution covered. The quality and stability of buffer may decrease according to pH measure and contamination. In the case of powder reagents, dissolve and divide into smaller parts, then store at -15 ~ -25 ℃.

3) Do not use more ethanol than the recommended amount. In addition, mark the total volume after adding ethanol. In order to prevent ethanol evaporation, close the cap tightly and store at 15 ~ 25 ℃.

4) All reagents and solution should be mixed completely before use. In addition, add buffer to each tube, then mix sufficiently.

5) Check out the pressure of vacuum pump. If the degree of vacuum is too high, the yield of recovery may decrease.

2. Low yield of DNA recovery after elution

1) When using inappropriate buffer (For instance: distilled water etc.) for elution or incorrect pH values, the yield of recovery may decrease. In general, when the pH value exceeds 8.5, the elution effect of is strong

3. White precipitates appear in Binding Buffer (B)

If the Binding Buffer (B) was store at lower temperature condition for a longer period, white precipitates will be formed in Binding Buffer (B),
In this case, the precipitates can be dissolved at 60 ℃. The precipitates have no effect on the performance capability of the kit.