USER GUIDE

AccuPrep® Genomic DNA Extraction Kit for 96 well Vacuum block

Cat. No. K-3032-2

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Kit for the extraction of Genomic DNA from bacterial cultures

User Guide

K-3032-2

Σ 192

Version No.: 3 (2022-03-22)

Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® Genomic DNA Extraction Kit for 96 well Vacuum block 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.

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.

Trademark

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Copyright

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

This kit contains adequate reagents for 192 (96 x 2) reactions.

Components	Amount	Storage
Proteinase K powder, lyophilized	25 mg x 4 ea	Refer to the “Storage” below.
GC Buffer (Binding)	50 ml	Store at room temperature (15-25°C).
WA1 Buffer (1st Washing)	40 ml x 2 ea	
W2 Buffer (2nd Washing)	80 ml x 2 ea	
EA Buffer (Elution)	60 ml	
96 well Binding plate	2 ea	
96 well dome plate	2 ea	
96 RV plate	2 ea	
96 well Sealing tape	20 ea	
One Page Protocol	1 ea	

Storage

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

All components of the kit should be stored dry at room temperature (15-25°C). Before use, lyophilized Proteinase K should be completely dissolved in 1,250 µl of nuclease-free water. For short term storage, dissolved Proteinase K should be stored at 4°C. For long term storage, it is recommended to aliquot these enzymes into separate tubes and store at -20°C.

*** Note:** Please note that repeated freezing and thawing may reduce its activity.

Specifications

Sample Type	Amount of Starting Sample	Typical DNA Yield	DNA Purity
Whole blood	200 µl	up to 3 µg	A_{260}/A_{280} > 1.8
Buffy coat	200 µl	up to 10 µg	
Cultured cells	10^4-10^8 cells	up to 10 µg	

*** Note:** There may be differences in measured values depending on the type of samples.

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because GC Buffer and WA1 Buffer contain chaotropic salts which are irritants.

Introduction

Product Description

AccuPrep® Genomic DNA Extraction Kit for 96 well Vacuum block is designed for extraction of highly purified genomic DNA from whole blood, leukocytes, or cultured cells using standard 96 well type plate. The kit employs 96 well Binding plate with glass fiber filter for nucleic acid binding in the presence of chaotropic salts. The DNA binds to the silica of glass fiber, while proteins and other contaminants are eliminated through a series of short wash-and-spin steps using ethanol. Finally genomic DNA is eluted by low salt solutions. The process does not require phenol/chloroform extraction and ethanol precipitation.

DNA extracted through this kit can be used for a variety of applications, including: gene cloning, PCR, Real-time PCR, southern blotting, and SNP genotyping.

Features & Benefits

- Comprehensive: High quality and yield of genomic DNA extraction from various samples such as whole blood or cultured cells.
- Efficient: Contaminants such as proteins and nucleases which may interfere with PCR reactions are completely removed.
- Minimized DNA damage: DNA damage is minimized by avoiding precipitation and use of organic solvents.
- Ready-to-use: Extracted DNA is ready-to-use for various application.

Experimental Procedures

Before You Begin

Before proceeding, please check the following:

1. Completely dissolve Proteinase K powder in 1,250 µl of nuclease-free water before use. Dissolved Proteinase K should be stored at 4°C.

2. Pre-heat EA Buffer at 60°C before use. This process is essential for maximal recovery in filter-based extraction.

3. Add indicated volume of absolute ethanol (not provided) to WA1 Buffer before use (see bottle label).

4. Adjust the degree of the vacuum of vacuum block. By putting tray in the vacuum block and equipping a new binding plate, you can measure the degree of the vacuum. Adjust the degree of the vacuum between 200 and 350 mmHg. If it is too high, the solution may be splashed, if it is too low, the filtering term may be longer. The method of adjusting the degree of the vacuum goes as follows: by putting tray at the bottom of vacuum block, you need put new or used binding plates on it. Add about 100 µl distilled water, and measure by producing vacuum. In the case of using new plates, it should be dried completely before use.

Protocol

1. Apply cultured cells (10^4-10^6 cells) or 200 µl of whole blood sample to a 96 well dome plate.
   * **Note:** If the sample volume is less than 200 µl, adjust the total volume to 200 µl by adding PBS buffer (not provided).

2. Add 20 µl of Proteinase K to the sample.

3. Add 200 µl of GC Buffer to the sample and seal the 96 well dome plate with a 96 well Sealing tape.
   * **Note:** Avoid wetting the rimes and cross-contamination.

4. Incubate at 60°C for 20 minutes.

5. Remove the 96 well Sealing tape and add 100 µl of isopropanol (not provided) to the lysate. Mix gently for 15 seconds.

6. Equip a waste tray at the bottom of the 96 well vacuum block and place the 96 well Binding plate on the top of it.

7. Transfer the cleared lysate from step 5 to the 96 well Binding plate.
   * **Note:** Avoid cross-contamination.

8. Filter completely with vacuum. Keep the vacuum at constant pressure and observe whether the solution passes through well.

9. Add 500 µl of WA1 Buffer to the 96 well Binding plate and filter with vacuum for 2-5 minutes.
   * **Note:** It is not necessary to completely remove ethanol in this step.

10. Remove the 96 well Binding plate and discard the solution obtained in the waste tray.
    * **Note:** If necessary, gently wipe off the bottom of the binding plate using paper towel.

11. Equip the waste tray at the bottom of the 96 well vacuum block and place the 96 well Binding plate on the top of it.

12. Add 700 µl of W2 Buffer to the 96 well Binding plate and filter with vacuum for 5 minutes.

13. Remove the 96 well Binding plate and discard the solution obtained in the waste tray.
    * **Note:** If necessary, gently wipe off the bottom of the binding plate using paper towel.

14. Shake off the 96 well Binding plate strongly and remove the moisture from the bottom of the binding plate. Tap and shake it off lightly until the moisture is wiped off.
    * **Note:** You should check out the remaining ethanol at the bottom of the binding plate.

15. Equip the waste tray at the bottom of the 96 well vacuum block and place the 96 well Binding plate on the top of it. Filter with vacuum for 5-10 minutes to remove the remaining ethanol completely.
    * **Note:** The remaining ethanol may influence the sequential steps after DNA extraction, so it is important to perform this step thoroughly.

16. Repeat step 14 once more.
    * **Note:** Mack sure whether there are small droplets hanging on each well of the bottom of the 96 well Binding plate or not, all droplets may be eliminated with a paper towel.

17. Place the 96 well RV plate at the bottom of the 96 well vacuum block and equip the 96 well Binding plate on the top of it. Add 200 µl EA Buffer and let stand for 1 minute.
    * **Note:** Pre-warmed EA Buffer will improve efficiency of elution (see “Before You Begin” on page 5).

18. Obtain the purified genomic DNA by vacuum.

Troubleshooting

Problem	Comments
Low DNA yield or purity	• **Buffers or other reagents may have been exposed to conditions that reduce their effectiveness.** Store the reagents at room temperature (15-25°C) at all times upon arrival and keep all reagent bottles tightly closed after use to preserve pH and stability and avoid contamination. After reconstitution of the lyophilized reagents, divide it into aliquots and store at -20°C.
	• **Ethanol may not have been added to WA1 Buffer.** Add indicated volume of absolute ethanol (not provided) to the WA1 Buffer (see “Before You Begin” on page 5) and mix well. Mark WA1 Buffer bottle label to indicate whether ethanol has been added or not.
	• **Reagents and samples may not have been completely mixed.** Always mix the sample tube well after adding each reagent.
	• **The pressure of vacuum pump may not have been appropriate.** Check out the pressure of vacuum pump. If the degree of vacuum is too high, the yield of recovery may decrease
	• **You may not have used optimal reagents for eluting nucleic acid.** An alkaline pH is required for optimal elution. Use EA Buffer included in the kit.
	• **The lysis may have been incomplete.** Mix the sample immediately after adding Proteinase K. Always mix the sample tube well with ethanol before loading the lysate into the binding column.
Incomplete or no restriction enzyme cleavage of isolated	• **Too much ethanol may have remained in the filtrate.** You should extend the drying time. If the problem still happens, completely dry the finally obtained genomic DNA and redissolve it

DNA	in distilled water before use.
	• **GC Buffer may not have been completely eliminated.** Repeat washing step with W2 Buffer once more.
Slightly colored final eluate from blood samples	• **The binding column may have been washed inadequately.** Wash the binding column until flow-through is colorless.
White precipitates in GC Buffer	• **Buffers may have been stored for prolonged periods of time at lower temperature.** Warm the buffers at 60°C and mix well to dissolve the precipitates.

Ordering Information

Description		Cat. No
AccuPrep® Genomic DNA Extraction Kit for 96 well Vacuum block	192 rxns (96 well x 2 ea)	K-3032-2

Related Products

Description	Cat. No
Proteinase K Powder	KB-0111
Phosphate Buffered Saline (PBS)	C-9024
BioVac™ 96 Vacuum Manifold	A-9030

Explanation of Symbols

LOT Batch Code	Consult Instructions For Use	RUO Research Use Only	Caution
Biological Risks	Contains Sufficient for <n> tests	Temperature Limitation	Manufacturer
REF Catalog Number	Do not Re-use	Use-by Date	

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