USER GUIDE

AccuPrep®
Genomic DNA Extraction Kit

Cat. No. K-3032

BIONEER
Innovation • Value • Discovery

AccuPrep® Genomic DNA Extraction Kit

Kit for the extraction of total DNA from whole blood, animal tissues, or cultured cells

User Guide

K-3032

Σ 100

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Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® Genomic DNA 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.

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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Contents

Product Information

Components

This kit contains adequate reagents for 100 reactions.

Components	Amount	Storage
Proteinase K powder, lyophilized	25 mg x 2 ea	Refer to the “Storage” below.
RNase A powder, lyophilized	24 mg x 2 ea	
TL Buffer (Tissue Lysis)	35 ml	Store at room temperature (15-25°C).
GB Buffer (Binding)	30 ml	
WA1 Buffer (1st Washing)	40 ml	
W2 Buffer (2nd Washing)	80 ml	
EA Buffer (Elution)	25 ml	
AccuPrep® Binding Column-I	100 ea	
Collection Tube (Filtration)	100 ea	
1.5 ml Tube (Elution)	100 ea	
One Page Protocol	1 ea	

Storage

The kit will maintain performance for at least three 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 and RNase A should be completely dissolved in 1,250 µl and 600 µl of nuclease-free water, respectively. For short term storage, dissolved Proteinase K and RNase A 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	3-6 µg	A_{260}/A_{280} > 1.8
Buffy coat	200 µl	15-20 µg	
Bacterial cells	< 10^9 cells	5-15 µg	
Cultured cells	10^4-10^6 cells	15-20 µg	
Animal tissue	25-50 mg	10-15 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 GB Buffer and WA1 Buffer contain chaotropic salts which are irritants.
- Thoroughly mix TL Buffer and GB Buffer by shaking before use.

Introduction

Product Description

AccuPrep® Genomic DNA Extraction Kit provides a rapid and convenient genomic DNA extraction method. *AccuPrep*® Genomic DNA Extraction Kit is designed for extraction of total DNA from whole blood, leukocytes, animal tissues, or cultured cells. The kit employs spin-column 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, animal tissues, or cultured cells.
- Convenient & Rapid: The process does not require phenol/chloroform extraction, ethanol precipitation, or other time-consuming steps.
- Efficient: Contaminants such as proteins and nucleases which may interfere with PCR reactions are completely removed.
- Ready-to-use: Extracted DNA is ready-to-use for various application.
- Minimized DNA damage: DNA damage is minimized by avoiding precipitation and use of organic solvents.
- High binding capacity: Uses silica-based DNA binding column with a high binding capacity of up to 30 µg.

Experimental Procedures

Materials to be Prepared by User

PBS buffer (Cat. No. C-9024)	137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4
Lysis buffer (for Gram-positive bacteria)	20 mM Tris-HCl, 2 mM sodium EDTA, 1.2% Triton® X-100, pH 8.0
Absolute ethanol	
Lysozyme (100 mg/ml)	

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. Completely dissolve RNase A powder in 600 µl of nuclease-free water before use. Dissolved RNase A should be stored at 4°C.

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

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

Preparing Lysates from Whole Blood and Buffy Coat

1. Apply 200 µl of whole blood or buffy coat sample to a clean 1.5 ml tube.

   ***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 GB Buffer to the sample and mix well by vortexing.

   ***Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

4. Incubate at 60°C for 10 minutes.

5. Add 400 µl of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysates clinging under the lid.

6. Proceed immediately to “Purification Procedure Using Spin Columns” on page 11.

Preparing Lysates from Cultured Cells

1. Harvest cultured cells (10^4-10^6 cells) by centrifugation at 300 x *g* for 5 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet.

2. Resuspend the cell pellet from step 1 in 200 µl of PBS buffer (not provided).

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

4. Add 10 µl of RNase A to the sample, gently mix, and incubate for 2 minutes at room temperature.

5. Add 200 µl of GB Buffer to the sample and mix well by vortexing.
   *** Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

6. Incubate at 60°C for 10 minutes.

7. Add 400 µl of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysate clinging under the lid.

8. Proceed immediately to “Purification Procedure Using Spin Columns” on page 11.

Preparing Lysates from Animal Tissue

1. Grind (or homogenize) 25-50 mg of fresh or thawed animal tissue sample with a mortar and pestle (or homogenizer) and place it into a clean 1.5 ml tube. Hard tissue can be ground to a fine powder in liquid nitrogen†.

   ***Note:** If the sample is not ground completely, it will result in significantly reduced DNA yields and clogging of the binding column.

   † After grinding, liquid nitrogen should be evaporated.

2. Add 200 µl of TL Buffer, 20 µl of Proteinase K, and 10 µl of RNase A to the sample from step 1 and mix well by vortexing.

   ***Note:** The sample should be completely immersed in the buffer.

3. Incubate at 60°C until the sample has been completely lysis (1 hour). The sample changes in clarity from turbid to clear, indicating that protein digestion has occurred.

   ***Note:** In general, it will take 1 hour until lysis is complete, but it may take more time depending on the type of tissue. For efficient lysis, you may perform shaking water bath or rocking platform. If these are not available, you should vortex 2-3 times every 30 minutes during incubation.

4. Add 200 µl of GB Buffer to the lysate and mix well by vortexing.

   ***Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

5. Add 400 µl of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysate clinging under the lid.

6. Proceed immediately “Purification Procedure Using Spin Columns” on page 11.

Preparing Lysates from Bacterial Cells (Gram-Negative Bacteria)

1. Harvest up to 1 x 10^9 bacterial cells by centrifugation at 8,000 rpm for 5 minutes to pellet cells. Discard the supernatant carefully with a pipette.

2. Resuspend the cell pellet from step 1 in 180 µl of TL Buffer by vortexing or pipetting. Transfer the cell suspension into a clean 1.5 ml or 2 ml tube.

3. Add 20 µl of Proteinase K and 10 µl of RNase A and mix well by vortexing.

4. Incubate at 60°C for 1 hour.

5. Add 200 µl of GB Buffer and mix well by vortexing.

7. Add 400 µl of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysate clinging under the lid.

8. Proceed immediately to “Purification Procedure Using Spin Columns” on page 11.

Preparing Lysates from Bacterial Cells (Gram-Positive Bacteria)

1. Harvest up to 1 x 10^9 bacterial cells by centrifugation at 8,000 rpm for 5 minutes to pellet cells. Discard the supernatant carefully with a pipette.

2. Resuspend the cell pellet from step 1 in 180 µl of lysis buffer (for gram-positive bacteria, not provided) by vortexing or pipetting. Transfer the cell suspension into a clean 1.5 ml or 2 ml tube.

3. Add 20 µl of lysozyme (100 mg/ml, not provided) and 10 µl of RNase A and mix well by vortexing.

4. Incubate at 37°C for 30 minutes.

5. Add 20 µl of Proteinase K and mix well by vortexing.

6. Add 200 µl of GB Buffer and mix well by vortexing.

7. Incubate at 60°C for 30 minutes or until bacterial cells are completely lysed.

8. Add 400 µl of absolute ethanol (not provided) to the lysate and mix well by pipetting. Briefly spin down to collect lysate clinging under the lid.

9. Proceed immediately to “Purification Procedure Using Spin Columns” on page 11.

Purification Procedure Using Spin Columns

1. Carefully apply the cleared lysate to the *AccuPrep*® Binding Column-I fit in a Collection Tube.

2. Centrifuge at 8,000 rpm for 1 minute and discard the flow through. Reuse the Collection Tube in step 3.
   * **Note:** You should put a cap on the column to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher than 10,000 rpm until the column is empty.

3. Wash the *AccuPrep*® Binding Column-I by adding 500 µl of WA1 Buffer.

4. Centrifuge at 8,000 rpm for 1 minute and discard the flow through. Reuse the Collection Tube in step 5.

5. Wash the *AccuPrep*® Binding Column-I by adding 500 µl of W2 Buffer.

6. Centrifuge at 8,000 rpm for 1 minute and discard the flow through. Reuse the Collection Tube in step 7.

7. Centrifuge once more at 13,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.
   * **Note:** Residual W2 Buffer in the binding column may cause problems in later applications.

8. Place the *AccuPrep*® Binding Column-I in a clean 1.5 ml Tube (supplied with a kit). Add 50-200 µl of EA Buffer or nuclease-free water to elute DNA.
   * **Note:** A smaller volume will result in a more concentrated solution, but total yield may be reduced. For long-term DNA storage, you should elute with EA Buffer and store at -20°C because DNA stored in water is subject to acid hydrolysis. Furthermore, pre-warmed EA Buffer will improve efficiency of elution (see “Before You Begin” on page 5).

9. Incubate at room temperature for 1 minute to be absorbed the EA Buffer completely into the glass fiber of the binding column.
   * **Note:** To increase DNA yield, you should incubate for 5 minutes after adding EA Buffer.

10. Centrifuge at 8,000 rpm for 1 minute.

11. To recover more DNA, repeat once more elution step using the eluate from step 10.

12. Centrifuge at 8,000 rpm for 1 minute.

***Note:** The eluted genomic DNA is stable and can be used directly or stored at 4°C for future experiment.

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.
	• **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.** Ensure that sample changes clarity from turbid to clear for occurring protein digestion. In general, it will take 1 hours until lysis is complete, but it may take more time depending on the type of tissue. For efficient lysis, you may perform shaking water bath or rocking platform (as directed in “Experimental Procedures”). Mix the sample immediately after adding Proteinase K. Always mix the sample tube well with ethanol before loading the lysate into the binding column.
	• **For tissue, lysis may not have been incomplete.** Cut the tissue into smaller pieces before digestion and lysis steps

	and extend incubation time. There are two ways to extend the incubation time with Proteinase K: a. Incubate tissue for overnight with Proteinase K. b. Incubate tissue with Proteinase K for 3-4 hours, then add 30 µl of a fresh aliquot of Proteinase K and incubate for another 1-2 hours.
	• **Glass fiber from binding column may have been eluted with nucleic acid.** These fibers can scatter light, resulting in a high absorbance value. In the last stage of elution, too much centrifugation could result in mixing the debris of glass fiber in the binding column into eluate. Please refer to the "Comments" below for instructions on removing the glass fibers.
Incomplete or no restriction enzyme cleavage of isolated DNA	• **Glass fiber from binding column may have been eluted with nucleic acid.** These fibers may inhibit enzyme reactions. After final elution step, centrifuge the tube at maximum speed for 1 minute. Glass fibers may be visible at the bottom of the tube. Transfer the supernatant to a new tube.
Degraded DNA from tissue samples	• **There may have been nuclease activity in the unlysed tissue.** Tissue should be frozen at -20°C immediately after harvesting and remained so until lysis step begins. Grind (or homogenize) tissue to a fine powder with mortar and pestle (or homogenizer) in liquid nitrogen.
Slightly colored final eluate from blood samples	• **The binding column may have been washed inadequately.** Wash the binding column until flow-through is colorless. Repeat purification step by mixing 200 µl of eluate with 200 µl of GB Buffer and 400 µl of absolute ethanol.
White precipitates in GB or TL 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	K-3032

Related Products

Description	Cat. No
Proteinase K Powder	KB-0111
RNase A Powder	KB-0101
Phosphate Buffered Saline (PBS)	C-9024

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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