User's Guide

AccuPrep®
Genomic DNA Extraction Kit

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AccuPrep® Genomic DNA Extraction Kit

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100

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Please read all the information in booklet before using the unit

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Safety Warnings and Precautions

For research use only

Not recommended for disease diagnose in 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.

Trademark

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I. Description

AccuPrep® Genomic DNA Extraction Kit can rapidly and conveniently extract an average of 6 μg of total DNA from a variety of sources, such as 200 μl of whole blood, 5 x 10^6 leukocytes, mammalian tissues (25 – 50 mg), or 10^4 – 10^8 cultured cells. *AccuPrep*® Genomic DNA Extraction Kit employs glass fibers, fixed in a column that specifically binds DNA in the presence of a chaotropic salt. Proteins and other contaminants are eliminated through a series of short wash-and-spin steps. Finally, the genomic DNA is eluted by a low salt solution. This process does not require phenol/chloroform extraction, alcohol precipitation, or other time-consuming steps. This kit is suitable to use with whole blood treated with either citrate or EDTA.

Advantages

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

Applications

1. Gene Cloning
2. PCR
3. Real time PCR
4. Southern Blotting
5. SNP genotyping

II. Kit components

This kit provides for 100 preparations and will maintain performance for at least two years under standard storage conditions.

Reagents	
Proteinase K, lyophilized	25 mg x 2 vials
One vial includes 25 mg of lyophilized Proteinase K. Dissolve this in 1.25 ml of nuclease-free water. Storage at −20℃ is recommended to prolong the activity of Proteinase K. Please note that repeated freezing and thawing may reduce its activity.	
RNase A, lyophilized	24 mg x 2 vials
One vial includes 24 mg of lyophilized RNase A. Dissolve this in 600 µl of nuclease-free water. Storage at −20℃ is recommended to prolong the activity of RNase A. Please note that repeated freezing and thawing may reduce its activity.	
TL Buffer	25 ml
Mix TL Buffer thoroughly by shaking before use. TL buffer is stable for 2 years when stored at room temperature (RT, 15 – 25℃).	
GB Buffer	25 ml
Mix GB Buffer thoroughly by shaking before use. GB buffer is stable for 2 years when stored at RT.	
* NOTE: Do not add lyophilized Proteinase K directly to GB buffer.	
WA1 Buffer	40 ml
WA1 Buffer is supplied in a concentrated form. Before the first use, add 30 ml of absolute ethanol. WA1 buffer is stable for 2 years when stored at RT.	
W2 Buffer	20 ml
W2 Buffer is stable for 2 years when stored at RT.	
EA Buffer	30 ml
10 mM Tris-HCl (pH 8.5). Store at room temperature.	
Columns and tubes	
Binding column	100 ea
Collection tubes (for filtration)	100 ea
1.5 ml tubes (for elution)	100 ea

Additional required materials

- Table-top microcentrifuge, 10,000 x g (13,000 rpm)
- Incubator, thermal block, or water bath
- Sterilized pipette tip
- Vortex mixer
- Absolute ethanol
- 1.5 ml or 2 ml tube (for preparation of lysate)
- Phosphate-Buffered Saline (PBS, C-9024)
- 20 mM Tris-HCl (pH8.0), 2 mM sodium EDTA and 1.2% Triton® X-100
- Lysozyme (100 mg/ml)
- Tissue homogenization tool (Scissor, mortar, Tissue Ruptor, Tissue Homogenization Set)

III. Before you begin

Before proceeding, please check the following:

1. Completely dissolve Proteinase K (KB-0111) in 1,250 μl of nuclease-free water. Dissolved Proteinase K should be stored at 4℃.

2. Completely dissolve RNase A (KB-3101) in 600 μl of nuclease-free water. Dissolved RNase A should be stored at 4℃.

3. Before starting the extraction process, you must preheat the solution EA to 60℃.

4. Add correct amount of absolute ethanol to WA1 Buffer.

☞ GB buffer contains irritant chaotrophic salt. You should take the appropriate laboratory safety precautions and wear gloves when handling.

☞ The g-force can be calculated as follows: $rcf = 1.12 \times r \times (rpm/1,000)^2$

IV. Experimental protocol

IV-1. DNA Extraction from Whole Blood and Buffy Coat

1. Add 20 µl of Proteinase K (see "Before you begin") to a clean 1.5 ml tube (see "Additional required materials").

2. Apply 200 µl of whole blood or buffy coat to the tube containing Proteinase K.
   If the sample volume is less than 200 µl, make the total volume 200 µl by adding PBS.

3. Add 200 µl of GB Buffer to the sample and mix immediately by vortex mixer.
   You must completely resuspend the sample to achieve maximum lysis efficiency.

4. Incubate at 60℃ for 10 min.

5. Add 400 µl of absolute ethanol and mix well by pipetting.
   After this step, briefly spin down to get the drops clinging under the lid.

6. Carefully transfer the lysate into the upper reservoir of the binding column tube (fit in a collection tube) without wetting the rim.

7. Close the tube and centrifuge at 8,000 rpm for 1 min.
   You must close each binding column tube to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed (>10,000 rpm) until the binding column tube is empty.

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

9. Add 500 µl of WA1 Buffer without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.

10. Discard the solution from the collection tube and reuse the collection tube.

11. Add 500 µl of W2 Buffer without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.

12. **Discard the solution from the collection tube and reuse the collection tube.**

13. **Centrifuge once more at 13,000 rpm for 1 min to remove ethanol completely, and check that there is no droplet clinging to the bottom of binding column tube.**
    Residual W2 in the binding column tube may cause problems in later applications.

14. **Transfer the binding column tube to a new 1.5 ml tube for elution (supplied), add 50 – 200 µl of EA Buffer (or nuclease-free water) onto binding column tube, and wait for at least 1 min at RT (15 – 25℃) until EA is completely absorbed into the glass fiber of binding column tube.**
    To increase DNA yield, you should wait for 5 min after adding EA Buffer. The volume of EA added can be adjusted from 50 to 200 µl. A smaller volume will result in a more concentrated solution, but total yield may be reduced.

15. **Centrifuge at 8,000 rpm for 1 min to elute.**
    About 180 – 200 µl of eluent can be obtained when using 200 µl of EA Buffer (or nuclease-free water). For an improved yield, elute the sample twice and use after concentration process.

    The eluted genomic DNA is stable and can be used directly, or stored at 4℃ for later analysis. For long-term DNA storage, you should elute with EA Buffer and store at -20℃, because DNA stored in water is subject to acid hydrolysis.

    About 6 µg of DNA in 200 µl of eluent (30 ng/µl) with an A_{260}/A_{280} ratio of 1.6 – 1.9 can be typically obtained from 200 µl of whole blood (~ 5 x 10^6 leukocytes/ml).

IV-2. DNA Extraction from Cultured Cells

1. Centrifuge the cultured cells (10^4 – 10^6) for 5 min at 300 x g. Discard the supernatant carefully without disturbing the pellet.

2. Resuspend the pellet in 200 µl of 1x PBS.

3. Add 20 µl of Proteinase K (see "Before you begin") to each tube.

4. Add 10 µl of RNase A (see "Before you begin") to each tube and incubate the tubes for 2 min at room temperature.

5. Go to step 3 of "DNA Extraction from Whole Blood and Buffy Coat" in page 4 and continue the instructions accordingly.

IV-3. DNA Extraction from Mammalian Tissue

1. **Disrupt (or homogenize) the sample (25 – 50 mg) with a mortar and pestle, place them in a clean 1.5 ml tube (see "Additional required materials"), and add 200 µl of TL Buffer.**

   Immediately place the weighted, fresh or frozen tissue in liquid nitrogen and grind to a fine powder with mortar and pestle under liquid nitrogen. Incomplete disruption will lead to significantly reduced yield and can cause clogging of the binding column tube. The final yield of DNA depends on the amount and the type of tissue used.

2. **Add 20 µl of Proteinase K and 10 µl of RNase A. Mix by vortex mixer.**

3. **Incubate at 60℃ for 1 hour, or until the tissue is completely lysed.**

   The sample changes in clarity from turbid to clear, indicating that protein digestion has occurred. The time required for lysis will vary depending on the type of tissue used. Lysis will usually take 1–3 hours, and for efficient lysis, a shaking water bath or rocking platform should be used. If these are not available, you should vortex 2–3 times, every 30 min during the incubation.

3. **Briefly spin down the tube to remove drops from the inside of the lid and add 200 µl of GB Buffer, and immediately mix by vortex mixer.**

   You must completely resuspend the sample to achieve maximum lysis efficiency.

4. **Go to step 5 of "DNA Extraction from Whole Blood and Buffy Coat" in page 4 and continue the extraction process.**

IV-4. DNA Extraction from Bacterial Cells (Gram-Negative Bacteria)

1. Collect the bacterial cells ~ 1 x 10^9 by centrifuging at 8,000 rpm for 5 min. Discard the supernatant (media) by using a pipette.

2. Add 180 µl of TL Buffer to the collected cell pellet and completely resuspend using a vortex mixer or by pipetting. Transfer the cell suspension to 1.5 ml or 2 ml tube.

3. Add 20 µl of Proteinase K and 10 µl of RNase A. Mix by vortex mixer.

4. Incubate at 60℃ for 1 hour.

5. Add 200 µl of GB Buffer, and immediately mix by vortex mixer.

6. Go to step 5 of "DNA Extraction from Whole Blood and Buffy Coat" in page 4 and continue the extraction process.

IV-5. DNA Extraction from Bacterial Cells (Gram-Positive Bacteria)

1. Collect the bacterial cells ~ 1 x 10^9 by centrifuging at 8,000 rpm for 5 min. Discard the supernatant (media) by using a pipette.
2. Add 180 µl of lysis buffer for Gram-Positive bacteria (not provided) to the collected cell pellet and completely resuspend using a vortex mixer or by pipetting.
   (Note) Lysis buffer for Gram-Positive bacteria can be prepared by using this formulation: 20 mM Tris-HCl (pH8.0), 2 mM sodium EDTA and 1.2% Triton® X-100
3. Transfer the cell suspension to 1.5 ml or 2 ml tube.
4. Add 20 µl of lysozyme (100 mg/ml, not provided) and 10 µl of RNase A to each tube and mix thoroughly using a vortex mixer.
5. Incubate the tubes at 37℃ for 30 min.
6. Add 20 µl of Proteinase K to each tube.
7. Add 200 µl of GB Buffer to each tube and mix thoroughly using a vortex mixer.
8. Incubate the tubes at 60℃ for 30 min or until bacterial cells are completely lysed.
9. Go to step 5 of "DNA Extraction from Whole Blood and Buffy Coat" in page 4 and continue the instructions accordingly.

V. Troubleshooting

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

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. After reconstitution of the lyophilized reagents, separate it into aliquots, and store the aliquots at –20℃.

2) Ethanol may not have been added to the WA1 Buffer.

After adding ethanol, mix WA1 Buffer well and always mark the WA1 Buffer bottles to indicate whether ethanol has been added or not.

3) Reagents and samples may not have been completely mixed.

Always mix the sample tube well after adding each reagent.

4) You may not have used the optimal reagent for eluting the DNA.

An alkaline pH is required for optimal elution. Use the EA Buffer included in the kit.

5) The lysis may have been incomplete.

Ensure that the sample changes clarity from turbid to clear, indicating that protein digestion has occurred. The time required for lysis will vary depending on the type of tissue used. Lysis will usually take 1 – 3 hours, and for efficient lysis, a shaking water bath should be used (as directed in the experimental protocol). Mix sample immediately after adding Proteinase K. Always mix the sample thoroughly with ethanol before adding the lysate onto the Binding column tube.

6) There is a low yield from tissue.

Ensure that the tissue was disrupted to small pieces (or a fine powder) before the digestion and lysis steps.

There are two following ways to increase 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 a fresh aliquot of Proteinase K (30 µl) and incubate for another 1 – 2 hours.

7) The absorbance (A_{260}) reading of product is too high.

The glass fibers from binding column tube may have eluted with nucleic acid. These fibers can scatter light, resulting in a higher absorbency reading. In the last stage of elution, too much centrifuge could result in mixing the debris of glass fiber in the binding column tube into the elution. See the comments in paragraph 2 below for instructions on removing the glass fibers.

2. There is an incomplete or no restriction enzyme cleavage of isolated DNA.

The glass fibers from binding column tube may have eluted with nucleic acid. These fibers may inhibit enzyme reactions.

After the final elution step has been completed, centrifuge at maximum speed for 1 min. Glass fibers may be visible at the bottom of the tube. Transfer the supernatant into a new tube, without disturbing the glass fibers at the bottom of the original tube.

3. DNA from tissue samples is degraded.

Tissue should be frozen (−20°C) immediately after harvesting and remain so until the lysis procedure starts. Tissue should be ground to a fine powder with mortar and pestle under liquid nitrogen. There may have been nuclease activity in the unlysed tissue.

4. The final eluent from blood sample is still slightly colored.

The binding column may have been washed inadequately. Wash the binding column tube until the flow through is colorless. Repeat the purification protocol by mixing 200 µl of eluent with 200 µl of GB Buffer, then 400 µl of ethanol.

5. There is a white precipitate in some buffer (TL or GB).

A white precipitate may form in TL Buffer or GB Buffer after prolonged storage at low temperature. Incubating at 60°C should dissolve any precipitate in buffer, TL or GB.

VI. 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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