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User's Guide ▶▶▶

Global Genomics Partner

AccuPrep[®] Genomic DNA Extraction Kit

Cat. No.: K-3032

BiONEER
bioneer corporation

V109E12

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For research use only.
Not recommended for disease diagnosis in humans or animals.

Wear gloves when you are handling skin irritants or harmful reagents.

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AccuPrep® Genomic DNA Extraction Kit

Technical Manual

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 200µl of whole blood, 5×10^6 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.

II. Kit Components

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

K-3032 AccuPrep® Genomic DNA Extraction Kit

Reagents

Proteinase K, lyophilized 25 mg X 2 vial
One vial with 25 mg of lyophilized proteinase K is included. Dissolve this in 1.25 ml of nuclease-free water. Storage at -20°C is recommended to prolong the activity of proteinase K. Please note that repeated freezing and thawing may reduce its activity.

Tissue Lysis buffer (TL) 25 ml
Mix TL buffer thoroughly by shaking before use. TL buffer is stable for 2 year when stored at room temperature (RT, 15-25°C).

Binding buffer (GC) 25 ml
Mix GC buffer thoroughly by shaking before use. GC buffer is stable for 2 year when stored at RT.

* NOTE: Do not add lyophilized Proteinase K directly to Binding buffer.

Washing buffer 1 (W1) 40 ml
W1 buffer is supplied in a concentrated form. Before the first use, add 30 ml of absolute ethanol. W1 buffer is stable for 2 year when stored at RT.

Washing buffer 2 (W2) 20 ml
W2 buffer is supplied in a concentrated form. Before the first use, add 80 ml of absolute ethanol. W2 buffer is stable for 2 year when stored at RT.

Elution buffer (EL) 30 ml
10 mM Tris-Cl (pH 8.5). Store at RT.

Columns and tubes

Binding column tubes	100 ea
2 ml tubes (for filtration)	100 ea
1.5 ml tubes (for elution)	100 ea

III. Additional Required Materials

1. Absolute ethanol
2. Absolute isopropanol
3. Table-top microcentrifuge, 10,000 xg (13,000 rpm)
4. Incubator, thermal block, or water bath (shaking)
5. Vortex mixer
6. 1.5 ml tube (for preparation of lysate)
7. Phosphate buffered saline (PBS)

IV. Before You Begin

Before proceeding, please check the following:

1. Did you dissolve proteinase K in 1.25 ml of nuclease-free water?
2. Did you add the correct amount of absolute ethanol to solution W1 and W2?
3. Before starting the extraction process, you must preheat the solution EL to 60°C.

☞ The buffer GC contains the irritant chaotropic salt. You should take the appropriate laboratory safety precautions and wear gloves when handling this.

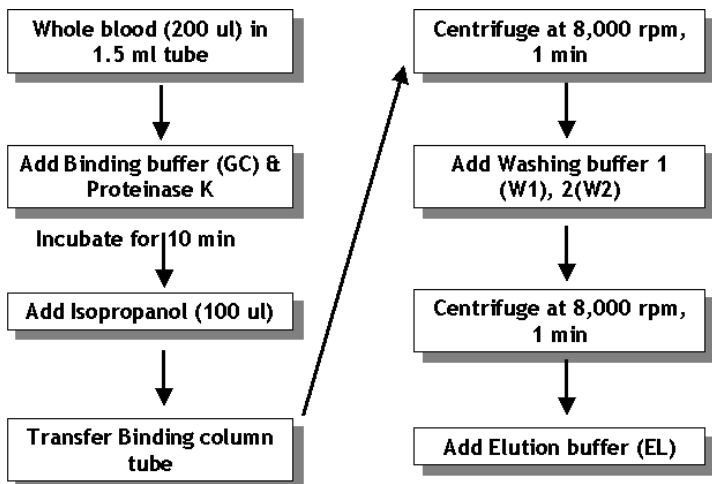
4. The g-force can be calculated as follows:

$$rcf = 1.12 \times r \times (\text{rpm}/1,000)^2$$

Where rcf is the relative centrifugal force (in g), r is the radius of the rotor in millimeters, and rpm is the speed of the centrifuge in revolutions per minute.

V. Experimental Protocol

V-1. Flow chart (for whole blood)



V-2. Isolation of DNA from whole blood, buffy coat, and cultured cells.

1. Add 20 μ l of Proteinase K (see “Before you begin”) to a clean 1.5 ml tube (see “Additional required materials”).
2. Apply 200 μ l of whole blood, buffy coat or 10^4 – 10^8 cultured cells to the tube containing proteinase K.
If the sample volume is less than 200 μ l, make the total volume 200 μ l by adding PBS.
3. Add 200 μ l of Binding buffer (GC) to the sample and mix immediately by vortex mixer.
You must completely resuspend the sample to achieve maximum lysis efficiency.
4. Incubate at 60°C for 10 min.
5. Add 100 μ l of Isopropanol and mix well by pipetting.
After this step, briefly spin down to get the drops clinging under the lid. Don’t vortex as this might reduce DNA yield.
6. Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a 2 ml 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. Open the tube and transfer the Binding column tube to a new 2 ml tube for filtration (supplied).

9. Add 500 μ l of Washing buffer 1 (W1) without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.
10. Open the tube and pour the solution from the 2 ml tube into a disposal bottle.
11. Carefully add 500 μ l of Washing buffer 2 (W2) without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.
12. Centrifuge once more at *ca.* 12,000 rpm for 1 min to completely remove ethanol, 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.
13. Transfer the Binding column tube to a new 1.5 ml tube for elution (supplied), add 200 μ l of Elution buffer (EL, or nuclease-free water) to Binding column tube, and wait for at least 1 min at RT (15~25°C) until EL is completely absorbed into the glass fiber of Binding column tube.
To increase DNA yield, wait for 5 min after adding Elution buffer (EL). The volume of EL added can be adjusted from 50 μ l to 100 μ l. A smaller volume will result in a more concentrated solution, but total yield may be reduced.
14. Centrifuge at 8,000 rpm for 1 min to elute.
About 180 μ l ~ 200 μ l of eluent can be obtained when using 200 μ l of Elution 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°C for later analysis. For long-term DNA storage, you should elute

with Elution buffer (EL) and store at -20°C , because DNA stored in water is subject to acid hydrolysis.

About $6\ \mu\text{g}$ of DNA in $200\ \mu\text{l}$ of eluent ($30\ \text{ng}/\mu\text{l}$) with an A_{260}/A_{280} ratio of 1.6 - 1.9 can be typically obtained from $200\ \mu\text{l}$ of whole blood ($\sim 5 \times 10^6$ leukocytes/ml).

V-3. Isolation of DNA 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 Tissue Lysis buffer (TL).**

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, mix by vortex mixer, and incubate at 60°C for 1 hr, 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 hr. A shaking water bath or rocking platform should be used for efficient lysis. If these are not available, 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 Binding buffer (GC), and immediately mix by vortex mixer.**
You must completely resuspend the sample to achieve maximum lysis efficiency.
- 4. Incubate at 60°C for 10 min.**
- 5. Add 100 µl of Isopropanol and mix well by pipetting.**
After this step, briefly spin down to get the drops clinging under the lid. Don't vortex as this might reduce DNA yield.
- 6. Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a 2 ml 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. Open the tube and transfer the Binding column tube to a new 2 ml tube for filtration (supplied).**
- 9. Add 500 µl of Washing buffer 1 (W1) without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.**
- 10. Open the tube and pour the solution from the 2 ml tube into a disposal bottle.**
- 11. Carefully add 500 µl of Washing buffer 2 (W2) without wetting the rim, close the tube, and centrifuge at 8,000 rpm for 1 min.**

- 12. Centrifuge once more at ca. 12,000 rpm for 1 min to completely remove ethanol, and check that there are no droplets clinging to the bottom of Binding column tube.**

Residual W2 in the Binding column tube may cause problems in later applications.

- 13. Transfer the Binding column tube to a new 1.5 ml tube for elution (supplied), add 200 µl of Elution buffer (EL, or nuclease-free water) to Binding column tube, and wait for at least 1 min at RT (15~25°C) until EL is completely absorbed into the glass fiber of Binding column tube.**

To increase DNA yield, wait for 5 min after adding Elution buffer (EL). The volume of EL added can be adjusted from 50 µl to 100 µl. A smaller volume will result in a more concentrated solution, but total yield may be reduced.

- 14. Centrifuge at 8,000 rpm for 1 min to elute.**

About 180 µl ~ 200 µl of eluent can be obtained when using 200 µl of Elution 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°C for later analysis. For long-term DNA storage, elute with Elution buffer (EL) and store at -20°C, 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 A260/A280 ratio of 1.6 ~ 1.9 can be typically obtained from 200 µl of whole blood (~ 5 X 10⁶ leukocytes/ml).

VI. 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 are stored at room temperature (15-25°C) at all times upon arrival, and that all reagent bottles are closed tightly after use to preserve pH, stability, and to avoid contamination. After reconstitution of the lyophilized reagents, separate them into aliquots, and store the aliquots at -20°C.

2) Ethanol may not have been added to the Washing buffer 1 and 2.

After adding ethanol, mix Washing buffers (W1 and W2) well and always mark the Washing 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 Elution buffer (EL) included in the kit.

5) The lysis may have been incomplete.

Ensure that the clarity of the sample changes 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 hr, 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 isopropanol before adding the lysate onto the Binding column tube.

6) There is a low yield from tissue.

Ensure that the tissue was pulverized to a fine powder, before the digestion and lysis steps.

There are two ways to increase the incubation time with Proteinase K:

- a. Incubate tissue overnight with Proteinase K.
- b. Incubate tissue with Proteinase K for 3 - 4 hr, then add a fresh aliquot of Proteinase K (30 μ l) and incubate for another 1 - 2 hr.

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 centrifuging could result in mixing the debris of the 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 Binding buffer (GC), then 100 µl of isopropanol.

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

A white precipitate may form in Tissue Lysis buffer (TL) or Binding buffer (GC) after prolonged storage at low temperature. Incubating at 60°C should dissolve any precipitate in buffer, TL or GC. The precipitate has no effect on function, and dissolving the precipitate at high temperatures will not improve yield nor quality of the purified nucleic acids.

VII. Supplementary material

1. Typical Results

The yield and purity of genomic DNA varies depending on the sample. The table below shows experimental results.

Sample	Amount of sample	Yield (μg)
Whole blood	200 μl	3 - 6
Cultured cells	10^6	15 - 20
Mammalian tissue	25 - 50 mg	10 - 15

Yields may vary between different blood donors, because they may have different amounts of leukocytes.

2. Yield of purified DNA

- 1) You can get about 6 μg of DNA in 200 μl of eluent (30 $\text{ng}/\mu\text{l}$) with an A_{260}/A_{280} ratio of 1.6 - 1.9, from 200 μl of whole blood ($\sim 5 \times 10^6$ leukocytes/ml).
- 2) The yield of DNA depends on both the amount and the type of tissue used. About 25 - 50 mg of tissue will yield approximately 15 - 20 μg of DNA in 200 μl of eluent (75 - 100 $\text{ng}/\mu\text{l}$), with an A_{260}/A_{280} ratio of 1.6 - 1.9.

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