Genomic DNA Isolation from Whole Blood Kit (KGI)

Introduction

Alpha Biobeads' blood DNA kit is designed to isolate nucleic acid from human whole blood. The magnetic nanoparticle (1 µm) technology enables efficient, consistent, and scalable extraction of genomic DNA from as little as 10 µL and up to 10mL blood. The obtained DNA is with high yield and purity (isolate 10 to 20 µg DNA from 350ul blood, A260/280 >1.7, A260/230 >1.8), and is suitable for direct downstream applications such as restriction enzyme digestion, PCR, qPCR and NGS. The magnetic particles feature uniformed size and slow sedimentation rate, which makes it an excellent choice for automation system applicable to high throughput sample preparation.

Kit Components and Storage

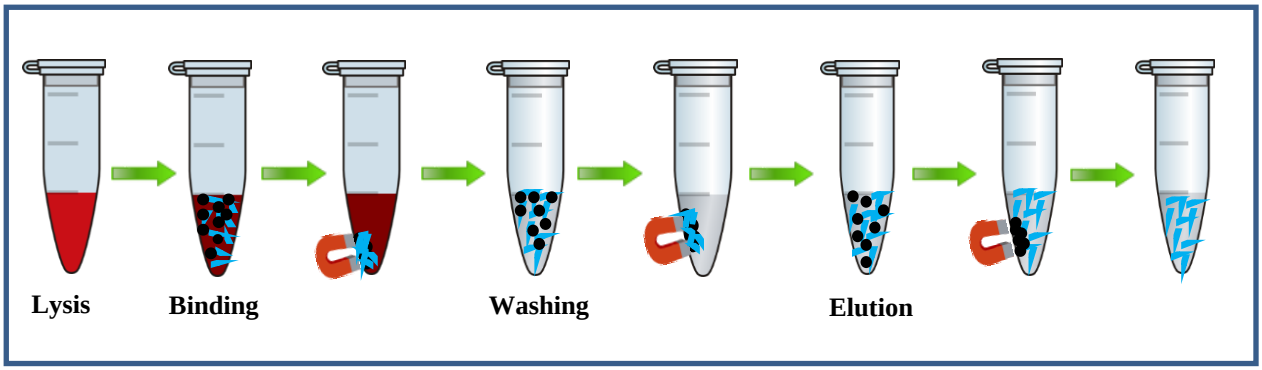
Each kit contains reagents for 96 reactions (based on 350µl blood/reaction):

Kit Components	Quantity	Storage
Magnetic Beads	2.5 mL (40 mg beads/mL)	2 to 8 °C, do not freeze
Lysis Buffer (GLB-40)	40 mL	2 to 8 °C
Washing Buffer 1 (GWB-1)	2 x 60 mL	2 to 8 °C
Washing Buffer 2 (GWB-2)	2 x 10 mL	2 to 8 °C
Elution Buffer (GEB-15)	15 mL	2 to 8 °C

Materials to be Supplied by the User

- Human Whole Blood (Fresh or Frozen)
- Proteinase K
- Isopropanol or 2-Propanol
- Ethanol
- Magnetic Separator

Protocol Flow Chart



Critical Notes Before You Start:

- Reconstitute Washing Buffer 1: Add 40 mL Isopropanol to the Washing Buffer 1 before your first use. Mix well and store at 2 to 8 °C.
- Reconstitute Washing Buffer 2: Add 90 mL Ethanol to the Washing Buffer 2 before your first use. Mix well and store at 2 to 8 °C.
- Prepare Proteinase K Solution (20 mg/mL): Dissolve Proteinase K powder in 10 mM Tris-HCl buffer (pH 8.0).
- The current protocol is for genomic DNA isolation from 350 µL human whole blood. The protocol has been validated to scale up or down to different blood volumes. If the amount of blood used has been changed, the amount of magnetic beads, proteinase K solution, lysis buffer, washing buffers, and elution buffer will need to be adjusted accordingly.
- There might be beads retained in the pipette tip during pipetting steps. Carefully pipette a few more times to release the beads back into the reaction in order to maximize yield.
- For any vortex steps, vortex at maximum speed to ensure mixing.

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Protocol

1. Add 50 µL Proteinase K solution (20 mg/mL) to a 350 µL blood sample in a 1.5 mL microcentrifuge tube. Vortex for 30 seconds and incubate for 2 minutes at room temperature.
2. Add 350 µL lysis buffer to the sample, and then vortex for 30 seconds to mix.
3. Incubate at 55°C for 5 minutes, and then vortex for 30 seconds to mix.
4. Repeat step #3 one more time.
5. Add 25 µL magnetic beads to the sample, and vortex for 15 seconds.
   Note: Please re-suspend the magnetic beads before adding the beads to the blood sample.
6. Add 500 µL isopropanol to the mixture, and vortex for 30 seconds to mix.
7. Incubate the mixture on a roller for 5 minutes, and vortex for 30 seconds to mix.
8. Place the tube on the magnetic separator. After 2 minutes, remove the supernatant using a pipette.
   Note: Please try to avoid touching the magnetic beads pellet with the pipette.
9. Remove the tube from the magnetic separator and add 1 mL Washing Buffer 1 to the pellet. Pipette up and down to fully resuspend the beads and then vortex for 30 seconds to mix.
10. Repeat steps #8 and #9 one more time.
11. Place the tube on the magnetic separator. After 2 minutes, remove the supernatant using a pipette.
12. Remove the tube from the magnetic separator and add 1 mL Washing Buffer 2 to the pellet. Pipette up and down to fully resuspend the beads. Vortex for 30 seconds to mix.
13. Use the pipette to transfer the magnetic beads to a clean tube.
14. Place the tube on a magnetic separator for 1 minute. Remove the supernatant. Avoid touching the pellet with the pipette.
15. Remove the tube from the magnetic separator and add 1 mL Washing Buffer 2 and vortex for 30 seconds. Place the tube on the magnetic separator for 1 minute. Remove the supernatant.
16. Leave the tube on the magnetic separator for 5 minutes to air dry.
    Note: Please remove any visible supernatant left in the tube with a small pipette tip. Don't let the pellet dry for over 5 minutes.
17. Take out the tube and add 100 µL Elution Buffer to the pellet and vortex for 2 minutes.
    Note: If it's difficult to resuspend the magnetic beads, a pipette could be used to pipette up and down to fully resuspend the beads.
18. Place the tube on the magnetic separator. After one minute, transfer the supernatant to a clean tube. This supernatant contains the purified genomic DNA.

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