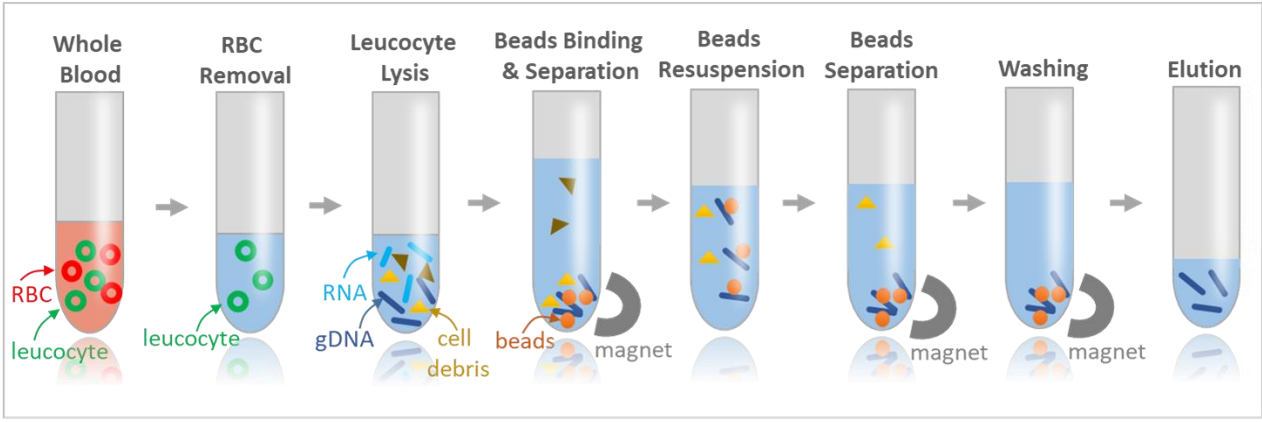
Genomic DNA Extraction Kit for Blood (HMW, Magnetic Beads)

Catalog No.	50015S	50015L
Preps	50 preps	200 preps

Description

The **Genomic DNA Extraction Kit for Blood (HMW, Magnetic Beads)** provides a reliable and fast process for extracting high molecular weight (HMW) genomic DNA from whole blood and buffy coat using Solid Phase Reversible Immobilization (SPRI) magnetic beads. With our proprietary magnetic beads technology, the kit eliminates the tedious centrifuge steps for columns. The kit provides a reliable and simple approach for high-quality genomic DNA isolation with fast magnetic response time and high binding capacity.

The extracted HMW genomic DNA size ranges are dependent on the beads resuspension: 50-150 kb by tube tapping and 40-100 kb by tube vortexing. Purified DNA is recovered at high yield and high purity without RNA contamination. The typical purity ratios of A260/A280 are around 1.8-2.0, and A260/A230 are around 2.2-2.5. Purified HMW genomic DNA is suitable for applications such as long-read sequencing, linked-read genome assembly, long range PCR, optical mapping, and other general applications.



Whole blood is resuspended in the RBC buffer to remove RBC. The remaining leucocytes are lysed with a lysis buffer, then mixed with beads to bind genomic DNA. The samples are mixed with a buffer and after washing steps, genomic DNA is eluted in Elution Buffer. The isolated genomic DNA with the magnetic beads is free of contamination such as RNA, proteins, salts, and other impurities.

Features

- High molecular weight DNA: 50 kb to 150 kb
- High purity
- Simple magnetic beads method
  - No column needed
  - No vacuum needed

	Specifications
Technology	Magnetic beads
Whole blood volume	Up to 200 µl
Genomic DNA size	50-150 kb (tube tapping) 40-100 kb (tube vortexing)
Genomic DNA yield	4-12 ug (200 µl blood)
Elution volume	100 µl
O.D. 260/280	1.8-2.0
O.D. 260/230	Above 2.0

Component

Catalog No.	50015S	50015L
RBC Buffer	80 ml	320 ml
TR Buffer	7.5 ml	30 ml
Lysis Buffer	12.5 ml	50 ml
gDNA Beads	7.5 ml	30 ml
RS Buffer	15 ml	60 ml
MB Buffer	12 ml	48 ml
Elution Buffer	5 ml	20 ml

Storage Condition

- Store gDNA Beads, RBC Buffer, TR Buffer, and MB Buffer at 4°C, stable up to 12 months.
- Store Lysis Buffer, RS Buffer, and Elution Buffer at room temperature, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- Vortexer
- 1.5 ml or 2.0 ml tubes
- 80% isopropanol (prepare before use)
- 80% ethanol (prepare before use)

Protocol

Part 1a, Whole blood samples (RBC removal)

Note: Up to 200 µl of whole blood can be processed. In the case of more than 200 µl of whole blood, split before processing. The high density of cells will make it difficult to resuspend DNA in solution.

A. Add 3X volume of RBC Buffer to blood samples (example: 600 µl of RBC Buffer to 200 µl of blood), invert or rock for 5 minutes at room temperature.
B. Centrifuge at 500 x g for 5 minutes at room temperature. Decant supernatant.
C. Add 1 mL of RBC Buffer, Invert or rock for 5-10 minutes until liquid is clear red. **Note**: Observe turbidity to evaluate lysis. Once the sample becomes clear, lysis is complete.
D. Centrifuge at 500 x g for 5 minutes at room temperature. Decant supernatant.
E. Add 150 µl of TR Buffer to the tube containing the cell pellet and mix by vortexing or pipetting.

Part 1b, Buffy coat samples

Note: Buffy coat from up to 200 µl of whole blood can be processed. In the case of a buffy coat sample from more than 200 µl of whole blood, split before processing. The high density of cells will make it difficult to resuspend DNA in solution.

A. Add 150 µl of TR Buffer to the tube containing the buffy coat sample and mix by vortexing or pipetting.

Part 2, Genomic DNA extraction

Note: Resuspend gDNA beads completely before use.

1. Add 250 µl of the Lysis Buffer to the above samples and mix by rotating the tube for 5 min.
2. Add 150 µl of the gDNA Beads, mix by inverting the tube 8 times and put the tube on a magnet rack immediately. Incubate for 4 min and carefully remove the supernatant using pipette tips. DO NOT touch the beads.
3. Add 300 µl of RS Buffer towards the opposite side of the beads, incubate 1 min, remove samples from the magnet, resuspend the beads with one of the following:
   a. Tapping or
   b. vortexing
   Note: It may take 5 to 30 min to fully resuspend the beads. The extracted DNA size ranges are dependent on the beads resuspension: 50-150 kb by tapping and 40-10 kb by vortexing.
4. Transfer samples to new tubes.
5. Add 240 µl of the MB Buffer, mix by inverting the tube 8 times and put the tube on a magnet rack immediately. Incubate for 4 min and carefully remove the supernatant using pipette tips. DO NOT touch the beads.
6. Add 800 µl of 80% isopropanol, remove samples from the magnet, resuspend the beads clumps in solution by vortexing briefly and gently. Invert the tube several times and incubate for 2 min. Make sure all the beads clumps are in the solution.
7. Put samples on the magnet and discard the supernatant.
8. Add 800 µl of 80% ethanol, remove samples from the magnet, resuspend the beads clumps in solution by vortexing briefly and gently. Invert the tube several times and incubate for 2 min. Make sure all the beads clumps are in the solution.
9. Put samples on the magnet and discard the supernatant. Dry the beads for 1 min and remove any residue solution using a pipette with fine tips.
10. Add 100 µl of Elution Buffer (10 mM Tris, pH 8.0), resuspend the beads by one of the following:
    a. tapping or
    b. vortexing
    Note: It may take 5 to 30 min to fully resuspend the beads. The extracted DNA size ranges are dependent on the beads resuspension: 50-150 kb by tapping and 40-10 kb by vortexing. DNA can also be resuspended in TE buffer or Low TE buffer.
11. Put the samples on the magnet and transfer the supernatant (containing DNA) to new tubes.

Troubleshooting

Low DNA concentration

1. Up to 200 µl of whole blood can be processed. In the case of more than 200 µl of whole blood samples, split before processing. The high density of cells will make it difficult to resuspend DNA in solution.
2. Resuspend gDNA beads completely before use.
3. Mix completely after adding the Lysis Buffer.
4. The gDNA Beads is viscous and make sure to add enough volume of gDNA Beads.
5. Make sure the beads are fully resuspended in the buffer (step 3 and 10).

RNA contamination

1. Extend incubation time from step 1 to 5-10 min.
2. Extend incubation time from step 2 to 5-10 min.

Low O.D. 260/230

1. Discard supernatant completely when washing (step 7 and 9).
2. Remove any residue solution using a pipette with fine tips at step 9.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.

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