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Cell/Blood Monarch HMW DNA Extraction Kit

Product Number: DNK52

Shipping and Storage

Transportation conditions: room temperature; Storage conditions: Store at 2-30℃.

Components

Component	10T	50T
Buffer FG1	35mL	175mL
Buffer Lysis A	2mL	10mL
Buffer Lysis B	2mL	10mL
Nucleic Acid Enhancer	1mL	5mL
Buffer AW2	3mL	15mL
Proteinase K（20 mg/mL）	0.14mL	0.7mL
RNase A（100 mg/mL）	0.07mL	0.35mL
Buffer TB	3mL	15mL
Nucleic acid beads	20 pieces	100 pieces
Container	10 of them	50 of them

Description

This kit is capable of extracting complete high molecular weight DNA from whole blood and cultured cell samples. The reagent kit innovatively uses optimized lysis buffer to specifically bind the extracted DNA A released from the sample to the surface of specially modified nucleic acid beads. The extraction time is as fast as 30 minutes (30 minutes for cell samples and about 60 minutes for blood samples), enabling rapid and efficient extraction of high molecular weight DNA. The extracted DNA length is greater than 50 kb and can reach up to Mb. The purified high molecular weight DNA has high yield and purity, and is suitable for various downstream applications such as third-generation long read sequencing, optical profiling technology, and chain read genome assembly.

Self provided instruments

1. Instrument: 4℃ microcentrifuge (with 2mL rotor), constant temperature shaker, horizontal mixer
2. Consumables: 1.5 and 2 mL centrifuge tubes (low adsorption, no DNase), wide mouth gun tip
3. Reagents: 10mM PBS, anhydrous ethanol (100%), isopropanol (AR).

Preparation and important precautions before the experiment

1. Blood samples should avoid repeated freezing and thawing, otherwise it may lead to a decrease in the quality and yield of extracted DNA
2. Buffer FG1 and PBS should be stored in a refrigerator at 4℃ before use.
3. Before the first use, anhydrous ethanol should be added to Buffer AW2 according to the instructions on the reagent bottle label.
4. Adjust the centrifuge to 4℃.
5. Adjust the temperature of the constant temperature shaker to 56℃.

Precautions for selecting starting materials

1. Blood sample

1.1. This kit can process fresh and frozen blood samples as well as blood samples treated with common anticoagulants (EDTA, citrate, heparin, and free DNA tubes). The extraction of blood samples begins with the step of red blood cell lysis, which

helps to obtain high yield, high purity, and high quality high molecular weight DNA. However, during the process of red blood cell lysis, the red blood cell lysate can cause some damage to white blood cells. Therefore, it is advisable to avoid prolonged exposure of white blood cells to red blood cell lysis buffer and to perform the red blood cell lysis step as soon as possible.

1.2. The recommended starting volume for the sample is 500μL. If the starting volume is>500μL, the sample will need to be initially processed in a larger tube or divided into several 500μL equal parts, and the container needs to hold 3 volumes of red blood cell lysis buffer.

2. Cell samples

2.1. This kit can extract high molecular weight DNA from fresh and frozen cultured cells (adherent or suspended).

2.2. The cell count should be controlled at 1×10^6, as excessive cell count can affect the lysis efficiency.

2.3. For the storage of cell samples, it is recommended to divide the required number of cells equally, centrifuge them into balls, freeze them in liquid nitrogen, and store them at -80℃. In contrast to slow freezing, fast freezing ensures that cell structure remains intact and is minimally affected by freezing.

Protocol

Blood sample

1. Red blood cell lysis

1.1. Take 500μL of fresh blood into a 2mL centrifuge tube (do not use a 1.5mL centrifuge tube), add 1.5mL of Buffer FG1 (3 times the sample volume), cover with a lid, gently invert 25-30 times, place on ice for 10 minutes, then invert and mix up after ice bath, and place on ice again for 3-5 minutes.

Note: Red blood cell lysate can cause certain damage to white blood cells; Prolonged exposure to leukocyte buffer for red blood cell lysis can lead to clumping, reduced yield, and decreased solubility of eluted DNA.

1.2. 1000 g, Centrifuge at 4℃ for 3 minutes, carefully discard the supernatant, retain 15-20μL of sediment, and place the centrifuge tube on ice.

Note: When absorbing the supernatant, do not touch the sediment and be sure to keep 15-20μL of liquid.

1.3. Add 1.5mL of Buffer FG1 and gently invert 8-10 times, then place it on ice for 3 minutes.

1.4. 1000 g, Centrifuge at 4℃ for 3 minutes, carefully discard the supernatant, retain 15-20μL of sediment, and place the centrifuge tube on ice.

Note: When absorbing the supernatant, do not touch the sediment and be sure to keep 15-20μL of liquid.

1.5. Add 1.5mL PBS and gently invert 8-10 times.

1.6. 1000 g, Centrifuge at 4℃ for 3 minutes, carefully discard the supernatant, retain 15-20μL of sediment, and place the centrifuge tube on ice.

Note: When absorbing the supernatant, do not touch the sediment and be sure to keep 15-20μL of liquid.

2. White blood cell lysis

2.1. Prepare cell lysis mixture 1 according to the table below

Cell lysis mixture 1	
Buffer Lysis A (μL)	RNase A (μL)
165	5.5

2.2. Add 150μL of cell lysis mixture (the mixture needs to be prepared and used immediately) to the centrifuge tube mentioned above, gently invert it 8-10 times, and let it stand at room temperature for 2 minutes.

2.3. Prepare cell lysis mixture 2 according to the table below

Cell lysis mixture 2	
Buffer Lysis B (μL)	Proteinase K (μL)
165	11

2.4. Add 150μL of cell lysis mixture (the mixture needs to be prepared and used immediately) to the centrifuge tube mentioned above, gently invert 8-10 times, and let it stand at 56℃ and 700 rpm for 10 minutes to completely dissolve the

precipitate.

2.5. Note: By adjusting the rotation speed, the length of the genome fragment can be changed. If the precipitate is not completely dissolved, the lysis time can be extended or the rotation speed of the metal bath can be increased until it is completely dissolved.

2.6. Add 75μL of Nucleic Acid Enhancer, gently invert 8-10 times, and let it stand at room temperature for 10 minutes.

3. DNA binding and elution

3.1. Add 2 nucleic acid beads and 275μL of isopropanol to the centrifuge tube using clean tweezers, and place it on a horizontal mixer at 20 rpm for 10 minutes. (Recommended by the manufacturer of the horizontal mixer: its Linbei SRT-303)

3.2. After completion, be careful to discard the supernatant.

Note: Do not touch Nucleic acid beads with the tip of the gun. Be sure to keep 30-40μL of liquid until the glass beads are exposed to the liquid level.

3.3. Add 500μL of Buffer AW2, place it on a horizontal mixer at 20 rpm for 2 minutes, and carefully aspirate and discard the supernatant.

Note: Do not touch Nucleic acid beads with the tip of the gun. Be sure to keep 30-40 μ L of liquid until the glass beads are exposed to the liquid level.

3.4. Repeat step 3.

3.5. Pour the aforementioned nucleic acid beads into a container containing 2mL centrifuge tubes and centrifuge for 1 second.

3.6. Pour the Nucleic acid beads from the above container into a new 2mL centrifuge tube, add 200μL Buffer TB, and let it stand at 56℃ and 300 rpm for 5 minutes.

Note: Nucleic acid beads should not be left in a dry state for a long time. Buffer TB can be added to a 2mL centrifuge tube in advance.

3.7. After completion, pour all the above solution (containing Nucleic acid beads) into a container containing 1.5mL centrifuge tubes and centrifuge at 12000 rpm for 30 seconds.

3.8. After completion, the visible nucleic acid precipitate is in a 1.5mL centrifuge tube. Later, it can be blown and mixed with a wide mouthed gun and stored in a -20℃ freezer for later use.

Cell samples

1. Red blood cell lysis

1.1. Take fresh cultured cells and suspend them in PBS solution in a 2mL centrifuge tube at 7000 rpm for 10 minutes. Remove the supernatant and place the centrifuge tube on ice.

Note: The cell count should be controlled at 1 × 106, as excessive cell count can affect the lysis efficiency.

1.2. Prepare cell lysis mixture 1 according to the table below.

Cell lysis mixture 1	
Buffer Lysis A (μL)	RNase A (μL)
165	5.5

1.3. Add 150μL of cell lysis mixture (the mixture needs to be prepared and used immediately) to the centrifuge tube mentioned above, gently invert it 8-10 times, and let it stand at room temperature for 2 minutes.

Note: Do not introduce bubbles.

1.4. Prepare cell lysis mixture 2 according to the table below.

Cell lysis mixture 2	
Buffer Lysis B (μL)	Proteinase K (μL)
165	11

1.5. Add 150μL of cell lysis mixture (the mixture needs to be prepared and used immediately) to the centrifuge tube mentioned above, gently invert 8-10 times, and let it stand at 56℃ and 700 rpm for 10 minutes to completely dissolve the precipitate.

Note: By adjusting the rotation speed, the length of the genome fragment can be changed. If the precipitate is not

completely dissolved, the lysis time can be extended or the rotation speed of the metal bath can be increased until it is completely dissolved.

1.6. Add 75μL of Nucleic Acid Enhancer, gently invert 8-10 times, and let it stand at room temperature for 10 minutes.

2. DNA binding and elution

2.1. Use clean tweezers to add 2 Nucleic acid beads and 275μL of isopropanol to the centrifuge tube mentioned above, and place it on a horizontal mixer at 20 rpm for 10 minutes (recommended by the horizontal mixer manufacturer: its Linbell SRT-303).

2.2. After completion, be careful to discard the supernatant.

Note: Do not touch Nucleic acid beads with the tip of the gun. Be sure to keep 30-40μL of liquid until the glass beads are exposed to the liquid level.

2.3. Add 500μL of Buffer AW2, place it on a horizontal mixer at 20 rpm for 2 minutes, and carefully aspirate and discard the supernatant.

Note: Do not touch Nucleic acid beads with the tip of the gun. Be sure to keep 30-40μL of liquid until the glass beads are exposed to the liquid level.

2.4. Repeat step 3 once.

2.5. Pour the aforementioned nucleic acid beads into a container containing 2mL centrifuge tubes and centrifuge for 1 second.

2.6. Pour the Nucleic acid beads from the above container into a new 2mL centrifuge tube, add 200μL Buffer TB, and let it stand at 56℃ and 300 rpm for 5 minutes.

Note: Nucleic acid beads should not be left in a dry state for a long time. Buffer TB can be added to a 2mL centrifuge tube in advance.

2.7. After completion, pour all the above solution (containing Nucleic acid beads) into a container containing 1.5mL centrifuge tubes and centrifuge at 12000 rpm for 30 seconds.

2.8. After completion, the visible nucleic acid precipitate is in a 1.5mL centrifuge tube. Later, it can be blown and mixed with a wide mouthed gun and placed in a -20℃ refrigerator.

Processing and storage of high molecular weight DNA samples

1. High molecular weight genomic DNA has a certain degree of viscosity, and gentle handling and the use of a wide caliber pipette tip will help maintain the integrity of DNA molecules. In addition, low adsorption tubes and suction heads should also be used.
2. The elution buffer Buffer TB provided by the reagent kit can be used as a buffer for long-term storage. If DNA samples are frequently used, it is recommended to store HMW samples at 4 ° C. If long-term storage is required, please store at -20 ° C and avoid repeated freezing and thawing as much as possible. Always use low adsorption tubes to prevent DNA from binding to the tube wall.