WIRAGEN
Molecular Research Tools

Blood WiraSpin®

DNA Extraction &Purification Kit

Ref : EA04

The WiraSpin® Human Blood DNA purification Kit provides a simple and convenient method for isolating high-quality genomic DNA using silica-based column technology. The kit is optimized for use with fresh or frozen blood samples ranging from 50 µL up to 200 µL in volume. It can be used with anticoagulated blood, dried blood spot, buccal swabs, saliva, serum, and leukocyte layer. The isolated DNA is suitable for PCR, qPCR, Southern Blot, enzymatic digestion, and sequencing applications.

Kit content

Components	20 rx	50 rx
Binding Buffer 4 (BB4)	4 ml	10 ml
Inhibitor Clean Buffer 4 (ICB4)	7 ml	15 ml
Wash Buffer 4 (WB4)	5 ml	10 ml
Elution Buffer 4 (EB4)	2 ml	5 ml
Lyophilized Proteinase K	10 mg	20 mg
Genomic Spin Columns with Collection Tubes	20	50

NB : Add 1 ml of ddH2O to the 20mg tube of Proteinase K or 0.5 ml of ddH2O to the 10 mg tube of Proteinase K and store at -20°C .

Operating Mode :

Before starting, add the specified volume of absolute ethanol to solutions ICB4 and WB4.

Components	20 rx	50 rx
Inhibitor Clean Buffer 4 (ICB4)	4,2 ml	9 ml
Wash Buffer 4 (WB4) per tube	20 ml	40 ml

All centrifugation steps are performed at room temperature.

Three specific protocols are delineated for the extraction of DNA, Including : **A- Whole Blood, Saliva, Serum; B- Buccal swab; and C- Dried Blood spot.** Common washing procedures are shared across all extraction methodologies.

- Protocols B and C require an additional volume of Binding Buffer (BB4). Supplementary Binding Buffer can be purchased separately.

A- Whole Blood, Saliva, Serum :

1. Place from 50 µl to 150 µl sample (Whole Blood, saliva, serum…) in a 1,5 mL microcentrifuge tube.

2. Add 20 µl of proteinase K (20 mg/ml) and 200 µl of Binding Buffer 4 (BB4) (for higher yield, preheat the buffer to 60 °C). Vortex for 15 seconds, then incubate at 56 °C for 15 minutes for Whole blood (note : if working with Saliva or Serum, incubate at 70 °C for 10 minutes).

3. Add 100 µl of Isopropanol, mix well, then transfer all lysate into the spin column silica. Centrifuge at 8000× g for 1 minute, then discard the filtrate.

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B- Buccal swab :

1. Place The buccal swab into a nuclease-free 1,5 mL microcentrifuge tube and cut it at the tube level. Add 500 µL PBS. Add 25 µL Proteinase K Solution and 400 µL Binding buffer 4 (BB4) to the microcentrifuge tube containing the buccal swab. Vortex the tube at maximum speed for 30 seconds. Incubate the tube at 65°C for 10 minutes. Discard the buccal swab.

2. Add 500 µL 100% ethanol to the tube. Vortex the tube at maximum speed for 20 seconds. Centrifuge briefly to collect any drops from the inside of the lid. Insert the spin column silica into a 2 mL Collection Tube. Transfer 750 µL of the mixture to the column. Centrifuge at 10,000g for 1 minute, discard the filtrate and re-use the collection tube.

3. Transfer the entire remaining mixture to the spin column silica. Centrifuge at ≥10,000g for 1 minute. Discard the filtrate. Insert the spin column silica into a new 2 mL Collection Tube.

C- Dried Blood spot :

1. Cut or punch-out the blood spot from the filter paper (up to 200 µL blood can be used per spot). Tear or cut the filter paper into small pieces and place them into a 1,5 mL microcentrifuge tube. Add 250 µL PBS. Incubate at 65°C for 1 hour. Vortex briefly every 20 minutes, add 25 µL Proteinase K Solution. Vortex at maximum speed for 15 seconds.

2. Incubate at 65°C for 30 minutes. Vortex briefly several times during incubation. Centrifuge at ≥13,000g for 5 minutes, transfer the supernatant to a 1,5 mL microcentrifuge tube. Add 1 volume of Binding Buffer (BB4) and 1 volume 100% ethanol. Vortex to mix thoroughly. Centrifuge briefly to collect any drops from the inside of the lid.

3. Insert a spin column silica into a 2 mL Collection Tube, transfer the entire sample to the column, centrifuge at ≥10,000g for 1 minute. Discard the filtrate, insert the spin column silica a new 2 mL Collection Tube.

Washing steps (Common to all samples)

4. Add 500 µl of Inhibitor Clean Buffer 4 (ICB4) (ensure ethanol has been added), centrifuge the column at 8000 × g for 1 minute, then discard the filtrate.

5. Add 500 µl of Wash Buffer 4 (WB4) (ensure ethanol has been added),centrifuge the column at 8000 × g for 1 minute, then discard the filtrate.

6. Repeat step 5 once.

7. Centrifuge the empty spin column at 13,000 × g for 10 seconds to remove any residual wash buffer. Air-dry the spin column at room temperature for several minutes.

8. Place the spin column in a clean 1.5 ml tube.

9. Add 30 to 100 µl of Elution Buffer 4 (EB4) directly into the center of the column (for higher yield, preheat the buffer to 70°C), let it sit at room temperature for 1 minute.

10. Centrifuge at 8000 × g 1 minute to elute the DNA. For long-term storage, store the DNA solution at -20°C.

Remarks:

- It is important not to overload the column, as this can result in significantly lower yields than expected.
- If you notice that the centrifugation spin column is overloaded, please repeat the centrifugation process.
- Use fresh materials and avoid repeated thawing/freezing cycles.
- Use sterile tubes and pipette tips to prevent RNase contamination.

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