

EasyPure® Blood Genomic DNA Kit

Cat. No. EE121

Storage: At room temperature (15-25°C) in a dry place for one year; RNase A at -20°C for two years.

Description

EasyPure[®] Blood Genomic DNA Kit provides a simple and convenient way to isolate high quality genomic DNA from 5-250 μl of fresh or frozen blood. Whole blood is incubated with binding/lysis buffer to release DNA. DNA is bound to silica-based column. The isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

- Simple and fast, red cell lysis buffer is no longer needed.
- Complete removal of contaminants and inhibitors.
- DNA yield up to 40 μg.
- Column based purification, no organic extraction or ethanol precipitation.
- Suitable for EDTA, sodium citrate and heparin-anticoagulated fresh or frozen blood in a volume of 5 to 250 μl.

Starting material

For blood with nonnucleated erythrocytes, we suggest using 5-250 µl; others, we suggest using 5-20 µl.

Kit Contents

Component	EE121-01 (50 rxns)	EE121-02 (200 rxns)		
Component	EE121-11 (50 rxns)	EE121-12 (200 rxns)		
Binding Buffer 3 (BB3)	30 ml	110 ml		
Clean Buffer 3 (CB3)	6 ml	24 ml		
Wash Buffer 3 (WB3)	12 ml	2×22 ml		
Elution Buffer (EB)	25ml	80 ml		
RNase A (20 mg/ml)	500 μl (EE121-01)	2×1 ml (EE121-02)		
Rivase A (20 mg/mi)	0 (EE121-11)	0 (EE121-12)		
Proteinase K (20 mg/ml)	1 ml	4×1 ml		
Genomic Spin Columns with Collection Tubes	50 each	200 each		

Procedures

Before starting, add the indicated volume of 96%-100% ethanol into the concentrated CB3 and WB3.

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Component	EE121-01/11 (50 rxns)	EE121-02/12 (200 rxns)
Clean Buffer 3 (CB3)	24 ml	96 ml
Wash Buffer 3 (WB3)	48 ml	2×88 ml

All centrifugation steps are carried out at room temperature.

- 1. Add the appropriate volume of blood, $20~\mu l$ of Proteinase K and $500~\mu l$ of BB3 into a microcentrifuge tube. Mix for 15 seconds by vortexing, and then incubate at room temperature for 10~minutes.
 - Optional: If RNA-free genomic DNA is required, add 20 µl of RNase A before incubation.
- 2. Centrifuge briefly, add all the lysate to a spin column. centrifuge at 12,000×g for 1 minute, discard the flow through.
- 3. Add 500 μ l of CB3 (check to make sure ethanol has been added), centrifuge the tube at 12,000 \times g for 30 seconds, discard flow through.
- 4. Add 500 μ l of WB3 (check to make sure ethanol has been added), centrifuge the tube at 12,000 \times g for 30 seconds, discard flow through.

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- 5. Repeat step 4 once.
- 6. Place the spin column to a collection tube. Centrifuge the empty column at 12,000×g for 2 minutes to remove any residual WB3. Air-dry the spin column at room temperature for several minutes.
- 7. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 µl of Elution Buffer (for higher yield, prewarm the buffer to 60°C) or distilled water (pH >7.0) to the center of column. Incubate at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute to elute the genomic DNA (to recover more DNA, add Elution Buffer or distilled water again to perform a second elution). For long-term storage, store the purified DNA at -20°C

Notes

- It is important not to overload the column, as this can lead to significantly lower yields than expected.
- · Use fresh material and avoid repeated thawing and freezing.
- Use sterile tubes and pipette tips to avoid the contamination from DNase.