

Blood DNA Preparation - Column Kit

DNA purification from blood with silica-membrane columns

Genomic DNA Preparation

Cat. No.	Amount
PP-237S	50 preparations
PP-237L	250 preparations

For in vitro use only

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Shelf Life: 12 months

Content

Component	PP-237S 50 preps	PP-237L 250 preps
Blood Lysis Buffer	52 ml	2 x 130 ml
Lysis Buffer	16 ml	80 ml
RNase A <u>store RNase A solution at -20°C</u>	7.5 mg add 150 µl ultra-pure water	5 x 7.5 mg add 150 µl ultra-pure water to each tube
Proteinase K <u>store Proteinase K solution at -20°C</u>	5 mg add 500 µl ultra-pure water	5 x 5 mg add 500 µl ultra-pure water to each tube
Binding Buffer	16 ml	80 ml
Activation Buffer	6 ml	30 ml
Washing Buffer	add 48 ml Ethanol (final volume 60 ml)	add 120 ml Ethanol to each bottle (final volume 150 ml)
Elution Buffer	5 ml	25 ml
Spin Columns	50	250
Collection Tubes	50	250

To be provided by you

- 96-99 % Ethanol
- ultra-pure water
- microtubes 1.5 or 2.0 ml
- heating block or water bath at 60 °C

Before start, add the following components (not included in the kit) as indicated in the **Content** table and/or on the bottle/tube:

- ultra-pure water to RNase A and Proteinase K (store soluted enzymes at -20°C)
- 96-99 % Ethanol to the Washing Buffer

Description

Blood DNA Preparation - Column Kit is designed for rapid and high purity isolation of genomic DNA from whole blood. Column-based purification completely removes PCR inhibitors such as divalent cations and proteins resulting in a high purity preparation of genomic DNA. There is no use of phenol or chloroform, handling is safe and does not produce harmful waste.

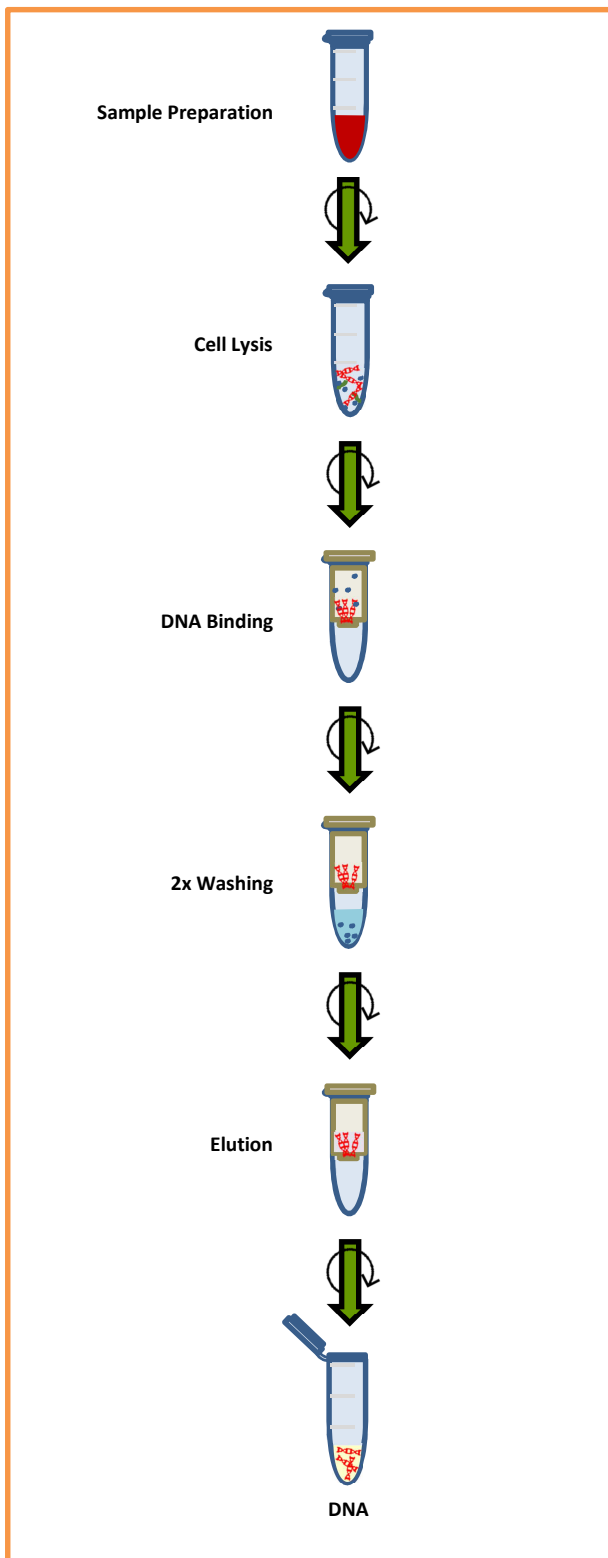
Application

The Blood DNA Preparation kit allows extraction of up to 10 µg of highly pure genomic DNA in a size range from 200 bp to 50 kb per preparation. The obtained DNA is suitable for a variety of applications, including real-time PCR, southern blot analysis, genotyping and discovery or validation of SNP/SSR markers.

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DNA Preparation

1) Sample Preparation

- Mix **200 µl (1 volume) of whole blood** with **1 ml (5 volumes) of Blood Lysis Buffer** in a microtube
- Incubate for 10 min on ice (vortex briefly 2-3 times during incubation)
- Centrifuge for 10 min at 10,000 g to pellet the white blood cells
- Discard the supernatant

2) Cell Lysis

- Add **300 µl Lysis Buffer** and **2 µl RNase A** to the cell pellet
- Vortex vigorously for 30 sec
- Incubate at 60 °C for 5 min
- Add **8 µl Proteinase K** and mix by pipetting
- Incubate at 60 °C for 10 min
- Cool down to room temperature

3) DNA Binding

- Add **300 µl Binding Buffer** and mix by inverting
- Place the tube on ice to cool down
- Centrifuge for 5 min at 10,000 g (repeat if the supernatant is not clear)
- Transfer the supernatant into a new tube
- Add **500 µl Ethanol (not included in the kit)**
- Mix by pipetting up and down

4) Column Activation

- Place a spin column into a 2 ml Collection Tube
- Add **100 µl Activation Buffer** into the Spin Column
- Centrifuge at 10,000 g for 30 sec
- Discard flow-through

5) Column Loading

- Pipet 600 µl supernatant from **DNA Binding** directly into the Spin Column (to avoid overloading of the column)
- Centrifuge for 1 min at 10,000 g
- Discard the flow-through
- Repeat the step with remaining supernatant

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6) Washing

- Add **500 µl Washing Buffer** into Spin Column
- Centrifuge for 30 sec at 10,000 g
- Discard the flow-through

Optional: Repeat Washing Step if highest purity is required

7) Remove residual Washing Buffer

- Centrifuge at 10,000 g for 2 min to remove residual Washing Buffer
- Discard the 2 ml Collection Tube
- Place the Spin Column into a new 1.5 ml microtube

8) Elution of DNA

- Add **50 µl Elution Buffer** into the center of the Spin Column
- Incubate at room temperature for 1 min
- Centrifuge at 10,000 g for 1 min
- DNA in Elution Buffer is stable at 4 °C or -20 °C for several months

Troubleshooting

Problem	Cause	Suggestions
Low yield	Insufficient lysis	Reduce the amount of starting material. Prolong the lysis step. Shake the sample during incubation.
	No Ethanol added to Washing Buffer	Make sure to add Ethanol to the Washing Buffer before start.
	DNA is sheared / degraded	Avoid excessive pipetting after lysis. Do not vortex after lysis.
	DNA is not completely eluted	Elute in two steps. Increase the volume of Elution Buffer. Prolong the incubation of the Elution Buffer on the Spin Column to 5 min. Make sure the Elution Buffer is delivered onto the matrix and not on the wall of the Spin Column.
RNA contamination	No / not enough RNase added	Make sure to add RNase A in the Cell Lysis step.
Inhibition of downstream enzymatic reactions	Ethanol carryover	Make sure to remove residual Washing Buffer before elution. Remove the Spin Column carefully from the Collection Tube so that it does not come in contact with the flow-through. If in doubt, centrifuge again for 1 min.