

# User's Manual and Instructions

## Blood and Serum DNA Isolation Kit

**Catalog Number: K5017100**

**Shipping Condition:** Room temperature

### Introduction

The Blood and Serum DNA Isolation Kit is designed for the purification of not only genomic DNA from whole blood or cells, but also genomic DNA fragment (circulating DNA) from serum, plasma, and bio fluid in a spin column format. No phenol-chloroform extraction, no Protease, and no precipitation steps are involved. The sample addition and washing steps can be performed using compatible vacuum manifold, while the lysis of cells and the final elution of the DNA product are performed using a table-top centrifuge. The blood cells in whole blood or other cultured cells and serum are first lysed in the lysis binding buffer that contains the denaturant guanidine HCl and Triton X100. Ethanol is then added to the samples, which are then added to the filter spin columns. This step facilitates the binding of DNA to the filter matrix. Under these conditions the DNA binds to the membrane while other contaminants are washed through. The columns are then washed to further remove protein, buffer components and other contaminants using two ethanol-containing wash buffers and the final genomic DNA product is eluted in TE. The final DNA product can be used directly for quantitative PCR and other downstream applications.

### Feature

- No phenol-chloroform
- No protease
- No precipitation
- Total <25 min.
- Sample range: 10 µl to 200 µl
- DNA yield: 3-9 µg/200 µl fresh whole blood

### Kit Contents

Item	Part #	Amount	Storage
1. Lysis Binding Buffer	K5017100-1	21 ml	RT
2. Wash Buffer 1	K5017100-2	26 ml	RT
3. Wash Buffer 2	K5017100-3	12 ml	RT
4. TE	K5017100-4	10 ml	RT
5. spin column set	K5017100-5	100 units	RT

### Storage Conditions

All of contents of the Blood DNA Isolation Kit including the buffers should be stored at room temperature. The kit is stable for one year under these conditions.

### Technical Assistance

Please refer any technical questions to [TechSupport@biochain.com](mailto:TechSupport@biochain.com).

### Important Notes Before Using The Blood DNA Isolation Kit

#### Sample Size and Type

The Blood and Serum DNA Isolation Kit can be used to isolate genomic DNA using a spin column format. DNA can be isolated quantitatively from less blood or cultured cells if required.

#### Buffer Concentrates

Wash buffers 1 and 2 are provided as concentrates that require the addition of 100% ethanol to them before use.

#### Reagents and Equipment to be Supplied by the User

- Pipetteman (multichannel pipettors desirable)
- 1.5 ml tubes

- Disposable gloves
- 100% ethanol
- Distilled deionized water
- A table-top centrifuge capable of providing >13k rpm rotor.

### Protocol

Before starting: The wash buffer 1 concentrate requires the addition of 26 ml of 100% ethanol before it can be used, while the wash buffer 2 concentrate requires that 48 ml of 100% ethanol is added to it before use. Both of the wash buffers are stable for one year after the addition of ethanol.

1. Transfer (up to) 200 µl of whole blood or cells or serum into a 1.5 ml tube. Add 200 µl of lysis binding buffer per tube. Pipette up and down until mix well, cap the tube and incubate at room temperature for 5-10 min (10 min. for serum).
2. Add 200 µl of 100% ethanol per tube to the samples containing binding buffer, pipette the plate well contents up and down twenty times to mix it well and add the contents to the filter column. After 10 min. incubation in the column, spin down the column at 13k rpm for one minute and discard the spin through liquid.
3. Wash the column by adding 500 µl wash buffer 1 (which contains the added ethanol) per column and spin the column as above condition.
4. Wash the column once by adding 600 µl wash buffer 2 (which contains the added ethanol) and spin the column as above.
5. Discard the liquid in the collection tube and spin the column one more minutes as above condition.
6. Place the column onto a new 1.5 ml tube for DNA sample elution. Add 100 µl of TE per column and wait for 5 min., and then centrifuge at 13k rpm for 1 min. to elute the final DNA product.

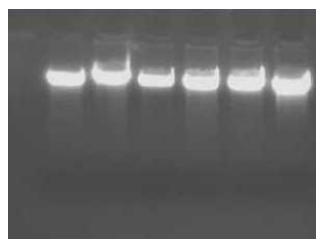
The use of 50 µl elution rather than 100 µl elution steps will provide you with a more concentrated DNA product, however the absolute yield of DNA will be reduced and the intrawell variation increased.

### Kit Performance

Table 1 shows the technical specifications of the DNA isolation kit.

TECHNICAL SPECIFICATIONS	
Yield	3-9 µg/200 µl fresh whole blood
Purity	UV <sub>260/280</sub> > 1.7
DNA size	99% of DNA is >20kb
Total time of prep	less than 25 min

Figure 1 shows the DNA quality on the 1% agarose gel.



### Related Products

- EZ-Blood DNA 96 Kit, Cat#. Z7040008
- EZ-DNA 96 Kit, Cat#. Z7040006
- Genomic DNA Extraction Kit, Cat# K5016005
- Serum DNA Isolation Kit, Cat# K5018100

**Trouble Shooting**

Problem	Comments and Suggestions
Little or no DNA eluted	Remove all traces of supernatant before beginning. All buffers must be at room temperature. Ensure that spin draws all liquid through filter membrane at each step. Measure final elution volume - ensure adequate final elution from final centrifugation steps. The more fresh blood gives better yield
Filters clog	Too much DNA/cells used. Reduce sample size.
Filters tear	Reduce centrifugation speed.

**Blood and Serum DNA Isolation Kit****Experienced Users Miniprotocol**

1. 200  $\mu$ l whole blood or serum & 200  $\mu$ l Lysis binding buffer, mix, 5-10 min (10 min. for serum) @ RT.
2. 200  $\mu$ l EtOH, mix well, add to column, incubate for 10 min., spin @13k rpm, 1 min.
3. 500  $\mu$ l wash buffer 1, spin @13k rpm, 1 min.
4. 600  $\mu$ l wash buffer 2, spin @13k rpm, 1 min.
5. spin @13k rpm, 1 min.
6. transfer the column onto a new 1.5 ml tube, 100  $\mu$ l of TE, incubate 5 min., spin @13k rpm, 1 min.

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