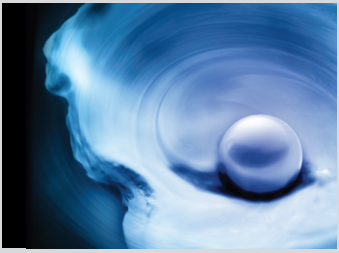


ISOLATE II
Blood DNA Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

ISOLATE II Blood DNA Kit

1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps	250 Preps
ISOLATE II Blood DNA Spin Columns (red) & Collection Tubes	10	50	250
Collection Tubes (2 mL)	20	100	500
Buffer G3	10 mL	15 mL	60 mL
Wash Buffer GW1	6 mL	30 mL	150 mL
Wash Buffer GW2† (concentrate)	6 mL	12 mL	50 mL
Elution Buffer G	13 mL	13 mL	60 mL
Proteinase K (lyophilized)	6 mg	30 mg	2 x 75 mg
Proteinase Buffer PR	1.8 mL	1.8 mL	8 mL
Product Manual	1	1	1
Bench Protocol Sheet	1	1	1

† Before use, add indicated volume of 96-100% ethanol and mark wash buffer bottle label.

2. DESCRIPTION

The ISOLATE II Blood DNA Kit is a simple, reliable and fast method for isolation of high-quality genomic DNA from a variety of sample sources including whole blood, cultured cells, serum, plasma or other body fluids.

Biological samples are first lysed in a buffer containing chaotropic salt ions in the presence of Proteinase K. Ethanol is added to the sample which is then processed through a Blood DNA Spin Column containing a silica membrane to which the genomic DNA binds. Any contaminants and impurities such as salts, metabolites and cellular components are effectively removed by simple washing steps with two different buffers. High-quality purified genomic DNA is then eluted in an elution buffer.

Please read this manual carefully to familiarize yourself with the ISOLATE II Blood DNA protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

3. STORAGE

Reconstituted Proteinase K solution is stable at -20°C for six months. All other kit components should be stored at room temperature (18-25°C) and are stable up to 1 year. Storage at lower temperatures may cause precipitation of salts.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Buffer GW1 contains guanidine hydrochloride. This chemical is harmful when in skin contact, inhaled or ingested.

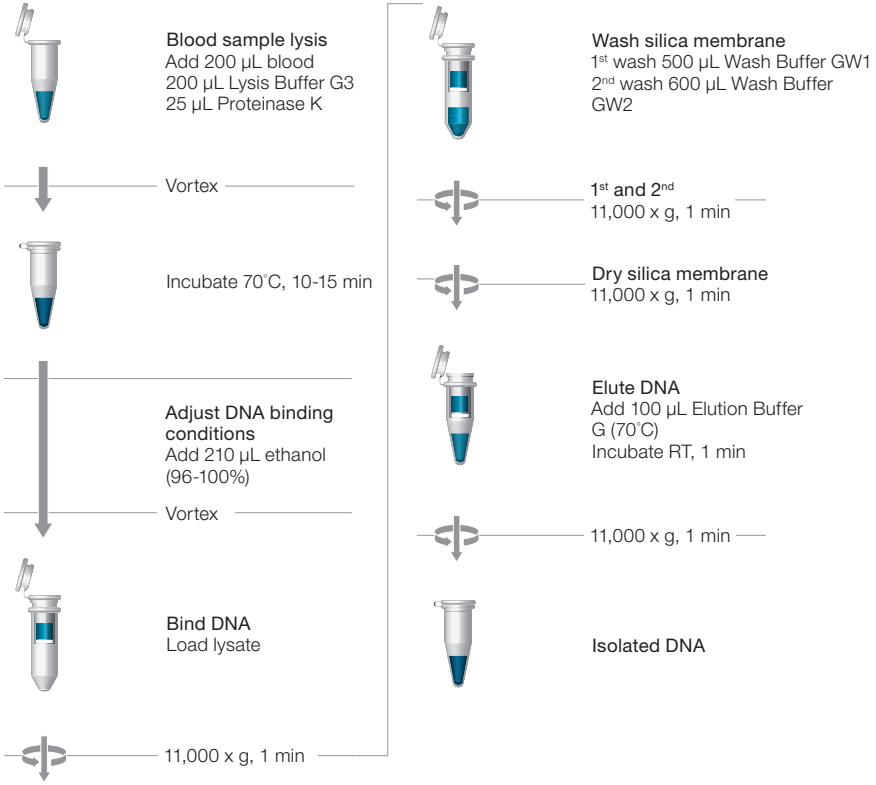
For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

The ISOLATE II Blood DNA Kit is specially designed for the rapid and efficient isolation of extremely pure genomic DNA, even from blood treated with EDTA, citrate or heparin. The kit is compatible with whole blood, serum, plasma or other body fluids samples, it is also possible to prepare pure viral DNA using cell-free samples (serum or plasma). The isolated DNA is of high purity (A_{260}/A_{280} ratio: 1.6-1.9) with yields of 40-60 ng/µL (see below).

ISOLATE II BLOOD DNA COLUMN SPECIFICATIONS	
Max. binding capacity	60 µg DNA
A_{260}/A_{280}	1.6-1.9
Typical yield	4-6 µg
Elution volume	100 µL
Max. amount of sample material	5 x 10^6 cells/200 µL

Blood DNA Isolation



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves.

- 96-100% ethanol†
- Microcentrifuge tubes (1.5 mL)
- Sterile DNase-free tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)
- Vortex mixer
- Thermal heating block

† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone.

7. IMPORTANT NOTES

7.1 HANDLING AND STORING STARTING MATERIALS

Blood samples

Blood samples stored at room temperature or +4°C for up to several days or weeks, will still allow DNA isolation. However, best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -70°C. Blood stored in this way is suitable for years for DNA isolation.

7.2 BUFFER PREPARATION AND PARAMETERS

Preparing Wash Buffer GW2

Add 96-100% ethanol to Wash Buffer GW2 Concentrate: 24 mL for the 10 prep kit, 48 mL for the 50 prep kit and 200 mL x 2 for the 250 prep kit.

Preparing Proteinase K Buffer PR

Add Proteinase K Buffer PR to the lyophilized Proteinase K: 260 µL for the 10 prep kit, 1.35 mL for the 50 prep kit and 3.35 mL x 2 for the 250 prep kit.

Note: Proteinase K solution is stable at -20°C for up to 6 months.

Elution parameters

It is possible to modify the elution protocol to improve yield and concentration.

Use Elution Buffer G preheated to 70°C for one of the following procedures:

- High yield: Two elution steps with 100 µL Elution Buffer G (to increase yield to 90-100%).

- High concentration: One elution step with 60 µL Elution Buffer G (to increase concentration by about 130%). Maximal yield of 80%.
- High yield and high concentration: Two elution steps. Add 50 µL Elution Buffer G, incubate for 3 min and centrifuge, repeat with a second 50 µL Elution Buffer G. Yield of 85-100% at a high concentration.

Store isolated DNA at -20°C. Several freeze-thaw cycles will not interfere with most downstream applications, however for long-range PCR or high sensitivity (especially in real-time PCR), store in aliquots to avoid multiple freeze-thaw cycles.

8. PROTOCOL

8.1 PURIFYING GENOMIC DNA FROM WHOLE BLOOD

Before you start:

- Make sure Wash Buffer GW2 and Proteinase K are prepared (see section 7.2).
- Set an incubator or water bath to 70°C.
- Preheat Elution Buffer G to 70°C.

1 **Lyse blood**

Add 25 µL Proteinase K solution and 200 µL sample into a 1.5 mL microcentrifuge tube (not supplied).

Note: Make up sample to 200 µL with PBS if using less volume. For cultured cells, resuspend up to 5 x 10^6 cells in 200 µL PBS.

Add 200 µL Lysis Buffer G3 and vortex vigorously for 10-20s.

Note: For high yield and DNA purity, vigorous mixing is essential.

Incubate samples at 70°C for 10-15 min.

Note: The lysate should turn brownish during incubation with Lysis Buffer G3. If processing older or clotted blood, increase Proteinase K incubation time up to 30 min and vortex vigorously several times during incubation.

2 **Adjust DNA binding conditions**

Add 210 µL ethanol (96-100%) and vortex.

3 **Bind DNA**

For each preparation, place one ISOLATE II Blood DNA Spin Column in a Collection Tube and load the sample onto the column. Ensure all lysate is loaded. Centrifuge for 1 min at 11,000 x g. Repeat at a higher g force if samples are not completely filtered through matrix. Place column in a new Collection Tube (2 mL).

4 **Wash silica membrane**

- Add 500 µL Wash Buffer GW1 and centrifuge for 1 min at 11,000 x g. Place column in a new Collection Tube (2 mL)
- Add 600 µL Wash Buffer GW2 and centrifuge 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.

5 **Dry silica membrane**

Centrifuge for 1 min at 11,000 x g to remove residual ethanol. Place the ISOLATE II Blood DNA Spin Column in a 1.5 mL microcentrifuge tube (not supplied).

6 **Elute DNA**

Add 100 µL preheated Elution Buffer G (70°C) directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge for 1 min at 11,000 x g.

Note: For alternative elution procedures see section 7.2.

9. TROUBLESHOOTING GUIDE

LOW DNA YIELD	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Low concentration of leukocytes	Use concentrated leukocytes in buffy coat instead of whole blood: centrifuge whole blood at room temperature (3,300 x g; 10 min) and use intermediate (buffy coat) layer.
Incomplete cell lysis	Sample must be vortexed vigorously immediately after addition of Lysis Buffer / Proteinase K solution. Proteinase K digestion not optimal: never add Proteinase K directly to Lysis Buffer. Increase cell lysis incubation time by an additional 15-20 min at 70°C. For old blood, clotting can occur: Mix vigorously once during the 70°C incubation step (section 8.1.1).
Reagents not applied correctly	Prepare buffers and Proteinase K solution according to instructions (section 7.2). Make sure ethanol is added to lysates before loading on columns (section 7.2). Reagents not stored optimally: store Proteinase K solution at -20°C. Store all other components at room temperature. Keep bottles tightly closed to prevent evaporation or contamination.
Suboptimal elution from the column	Apply preheated (70°C) Elution Buffer G directly onto the center of the silica membrane. If not using Elution Buffer G, make sure Elution Buffer used is slightly alkaline (pH 8.5).
POOR DNA QUALITY	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incomplete cell lysis	Sample must be vortexed vigorously immediately after addition of Lysis Buffer G3 / Proteinase K solution. Decreased Proteinase K activity: Store dissolved Proteinase K at -20°C for 6 months. Add more Proteinase K solution to sample and incubate up to 30 min, vortexing vigorously.
Reagents not applied correctly	Prepare buffers and Proteinase K solution according to instructions (section 7.2). Make sure ethanol is added to lysates before loading on columns (section 7.2).
RNA in sample	To remove RNA add 20 µL RNase A solution (20 mg/mL) before addition of lysis buffer.
Old or clotted blood samples processed	For older or clotted blood samples, prolong Proteinase K incubation to 30 min and vortex several times.

SUBOPTIMAL PERFORMANCE OF EXTRACTED GENOMIC DNA IN ENZYMATIC REACTIONS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Ethanol carry-over	Be sure to remove all traces of Wash Buffer GW2 before eluting the DNA. If necessary repeat silica membrane drying step a second time.
Contamination of DNA with inhibitory substances	We recommend elution with Elution Buffer G, as chemicals such as EDTA can interfere with downstream applications.
	If preparing DNA from older or clotted blood samples, extend Proteinase K incubation step to 30 min, vortexing once or twice.
	If the A_{260}/A_{280} ratio of the eluate is below 1.6, repeat the purification procedure: Add equal volumes of Lysis Buffer G3 and ethanol to the eluate, load column and proceed with step 3 of the protocol.

A. TECHNICAL SUPPORT

For technical assistance or more information on this product, please email us at tech@bioline.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Blood DNA Kit	10 Preps	BIO-52062
ISOLATE II Blood DNA Kit	50 Preps	BIO-52063
ISOLATE II Blood DNA Kit	250 Preps	BIO-52064

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Genomic DNA Kit	10 Preps	BIO-52065
ISOLATE II Genomic DNA Kit	50 Preps	BIO-52066
MyTaq™ HS DNA Polymerase	250 Units	BIO-21111
SensiFAST™ SYBR No-ROX Kit	200 Reactions	BIO-98002

D. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace free of charge any product that does not conform to the specifications. This warranty limits Bioline's liability only to the replacement of the product.

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