



## GRS Genomic DNA Kit – Blood & Cultured Cells – #GK02.0100 (100 preps) (FOR RESEARCH ONLY)

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<b>Sample :</b>	up to 300µl of whole blood, up to 200µl of frozen blood, up to 200µl of buffy coat, up to 1x10 <sup>7</sup> cultured animal cells.
<b>Expected Yield :</b>	up to 50µg DNA
<b>Format :</b>	spin column
<b>Operation Time :</b>	within 60 minutes
<b>Elution Volume :</b>	50-200 µl

### Product Description

The GRS Genomic DNA Kit – Blood & Cultured Cells - provides an efficient and fast method for the purification and or concentration of high quality total DNA (including genomic DNA, mitochondrial DNA and viral DNA) from a variety of samples, including whole and frozen blood, buffy coat and cultured animal cells. Eluted purified DNA (approximately 20-30kb) is suitable for PCR, and other enzymatic reactions.

### Principle

The GRS Genomic DNA Kit – Blood & Cultured Cells - uses chaotropic salts to lyse cells and to denature proteins. The buffer system is optimized to allow selective binding of DNA to the glass fiber matrix of the spin column (1). Contaminants such as proteins, divalent cations, unincorporated nucleotides, and enzyme inhibitors are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified genomic DNA is subsequently eluted by a low salt Elution Buffer or TE or water. The entire procedure can be completed within an hour without phenol/chloroform extraction or alcohol precipitation, with typical DNA yields of 6µg from 200µl of whole human blood or up to 50µg from 200µl of buffy coat.

### Quality Control

The quality of the GRS Genomic DNA Kit – Blood & Cultured Cells - is tested on a lot-to-lot basis by isolating total DNA from 200µl of whole human blood. Purified DNA quantified using a spectrophotometer with a typical yield of 4-6µg of genomic DNA with an A260nm/A280nm ratio of 1.6-1.8) and further checked by agarose gel electrophoresis.

### Caution

Buffer BC1 contains guanidine hydrochloride which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

### References

(1) Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615-619

**Kit Contents (100 preps)**

Red Blood Cell Lysis Buffer	135 ml
Buffer BC1	40 ml
Buffer BC2	30 ml
Wash Buffer 1	45 ml
Wash Buffer 2*	25 ml
Elution Buffer	30 ml
RNase A (10mg/ml)	0,55 ml
Proteinase K (3x11mg)**	33 mg
Genomic DNA mini spin column	100
2,0-ml collection tube	200
1,5-ml microtube (DNase/RNase free)	200

**Required/Optional Components (not included)**

Ethanol (96%-100%)
Centrifuge for microtubes
Pipets (and tips)
Vortex
Water bath or Thermoblock
PBS <sup>1</sup>

**Notes**

\* Add 100 ml ethanol (96%-100%) [not included] to Wash Buffer 2 prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed.

\*\* Add 1,1ml of ddH<sub>2</sub>O to each vial of Proteinase K and store at 4°C.

**Storage**

RNase A (10mg/ml) should be stored at -20°C and Proteinase K (11mg) at 4°C. All other components should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37°C followed by cooling to 25°C.

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<sup>1</sup> PBS (Phosphate Buffered Saline) = 137 mM NaCl; 2.7 mM KCl; 10 mM Na<sub>2</sub>HPO<sub>4</sub>; 2.0 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4

## PROTOCOL FOR DNA PURIFICATION FROM FRESH BLOOD

[Red Blood Cell Lysis Buffer Treatment removes non-nucleated red blood cells and reduces hemoglobin contamination. If the blood sample is less than 5µl or if the sample consists of nucleated blood cells (e.g. avian blood), it is recommended to use the Cultured Cells Protocol (page 6) to purify genomic DNA].

- 1) Transfer up to **300µl** of fresh blood (from a blood collection tube containing EDTA or other anticoagulant) to a 1,5-ml microcentrifuge tube, and add 3 volumes of **Red Blood Cell Lysis Buffer**. Mix by inversion. **DO NOT VORTEX!** (e.g. add 600µl of the lysis buffer to 200µl of fresh blood).
- 2) Incubate at **room temperature** for **10 minutes**.
- 3) Centrifuge for 5 minutes at 3.000g and remove the supernatant completely. Resuspend the pellet with **100µl of Red Blood Cell Lysis Buffer** and add **200µl of Buffer BC1**. Mix well by shaking vigorously.
- 4) Incubate during 10 minutes at **65°C-70°C** or until the sample lysate is clear. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70°C water bath to be used in step 11].
- 5) [Optional step; If RNA-free DNA is required] Allow the mixture to cool to room temperature and add 5µl of **RNase A (10mg/ml)**, vortex, and incubate for 5 minutes at room temperature.
- 6) Add 200µl of absolute ethanol to the lysate and mix by shaking vigorously immediately for 10 seconds. In case precipitate appears, break it up by pipetting.
- 7) Place the **genomic DNA mini spin column** in a 2-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.
- 8) Centrifuge at 14.000g-16.000g for 5 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.
- 9) Add 400µl of **Wash Buffer 1** and centrifuge at 14.000g-16.000g for 30 seconds. Discard the flow-through and place the genomic DNA mini spin column back in the collection tube. Add 600µl of **Wash Buffer 2\*** and centrifuge at 14.000g-16.000g for 30 seconds (\*check if ethanol is added first time the kit is used; see Notes on page 2).
- 10) Discard the flow-through and place the genomic DNA mini spin column back in the collection tube and centrifuge for another 3 minutes at 14.000g-16.000g to dry the matrix of the column.
- 11) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 100µl **preheated Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3-5 minutes.  
**Notes:** Instead of Elution Buffer, DNA can also be diluted with TE or water; pH should be 8.0-8.5. Standard elution volume is 100µl. To increase concentration elute with 30-50µl. To increase yield, elute with 200µl.
- 12) Centrifuge for 30 seconds at 14.000g-16.000g to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20°C.



## PROTOCOL FOR DNA PURIFICATION FROM FROZEN BLOOD

0) Transfer up to **200µl of blood** (if the sample volume is less than 200µl, add PBS to a final volume of 200µl) to a 1,5-ml microcentrifuge tube, and add **30µl of Proteinase K** (10mg/ml). Mix by inversion.

1) Incubate at **60°C** for 15 minutes.

2) Add **200µl of Buffer BC1**. Mix by shaking vigorously, and incubate at **70°C** for 15 minutes. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70°C water bath to be used in step 11].

3) [*Optional step; If RNA-free DNA is required*] Allow the mixture to cool to room temperature and add 5µl of **RNase A (10mg/ml)**, vortex, and incubate for 5 minutes at room temperature.

4) Add 200µl of absolute ethanol to the lysate and mix immediately for 10 seconds by shaking vigorously. In case precipitate appears, break it up by pipetting.

5) Place the **genomic DNA mini spin column** in a 2-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.

6) Centrifuge at 14.000g-16.000g for 5 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.

7) Add **400µl of Wash Buffer 1** and centrifuge at 14.000g-16.000g for 30 seconds. Discard the flow-through and place the genomic DNA mini spin column back in the collection tube.

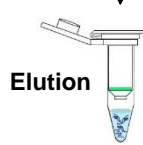
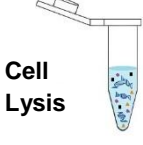
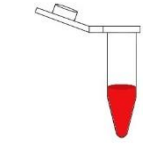
8) Add **600µl of Wash Buffer 2\*** and centrifuge at 14.000g-16.000g for 30 seconds (**\*check if ethanol is added first time the kit is used; see Notes on page 2**).

9) Discard the flow-through and place the genomic DNA mini spin column back in the collection tube and centrifuge for another 3 minutes at 14.000g-16.000g to dry the matrix of the column.

10) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 100µl **preheated Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3-5 minutes.

**Notes:** Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5. Standard elution volume is 100µl. To increase concentration elute with 30-50µl. To increase yield, elute with 200µl.

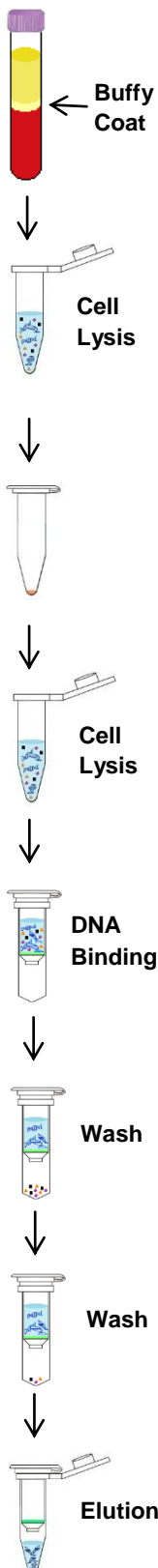
11) Centrifuge for 30 seconds at 14.000g-16.000g to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20°C.



## PROTOCOL FOR DNA PURIFICATION FROM BUFFY COAT

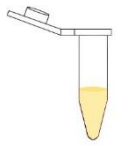
[Red Blood Cell Lysis Buffer Treatment removes non-nucleated red blood cells and reduces hemoglobin contamination. If the blood sample is less than 5µl or if the sample consists of nucleated blood cells (e.g. avian blood), it is recommended to use the Cultured Cells Protocol (page 6) to purify genomic DNA].

- 1) Transfer up to **200µl** of buffy coat to a 1,5-ml microcentrifuge tube, and add 3 volumes of **Red Blood Cells Lysis Buffer**. Mix by inversion and incubate for 10 minutes at room temperature. During incubation, invert the tube regularly (e.g. add 600µl of the red blood cell lysis buffer to 200µl of buffy coat).
- 2) Centrifuge for 1 minute at 14.000g-16.000g and remove the supernatant completely. Resuspend the white pellet with **500µl of Red Blood Cell Lysis Buffer**.
- 3) Centrifuge for 1 minute at 14.000g-16.000g and remove the supernatant completely. Resuspend the white pellet with **200µl of Red Blood Cell Lysis Buffer** and add **250µl of Buffer BC1**. Mix well by shaking vigorously.
- 4) Incubate at 65°C-70°C for 30 minutes or until the sample lysate is clear. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70°C water bath to be used in step 12].
- 5) [Optional step; If RNA-free DNA is required] Allow the mixture to cool to room temperature and add 5µl of **RNase A (10mg/ml)**, vortex, and incubate for 5 minutes at room temperature.
- 6) Add 250µl of absolute ethanol to the lysate and mix immediately by shaking vigorously for 10 seconds. In case precipitate appears, break it up by pipetting.
- 7) Place the **genomic DNA mini spin column** in a 2-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.
- 8) Centrifuge at 14.000g-16.000g for 5 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.
- 9) Add **400µl of Wash Buffer 1** and centrifuge at 14.000g-16.000g for 1 minute. Discard the flow-through and place the genomic DNA mini spin column back in the collection tube. Add **600µl of Wash Buffer 2\*** and centrifuge at 14.000g-16.000g for 1 minute (**\*check if ethanol is added first time the kit is used; see Notes on page 2**).
- 10) Discard the flow-through and place the genomic DNA mini spin column back in the collection tube and centrifuge for another 3 minutes at 14.000g-16.000g to dry the matrix of the column.
- 11) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 100µl **preheated Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at **37°C** (e.g. incubator) for 10 minutes.  
**Notes:** Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5. To increase DNA concentration elute with 30-50µl. To increase overall yield, elute with 200µl.
- 12) Centrifuge for 1 minute at 14.000g-16.000g to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20°C.



## PROTOCOL FOR DNA PURIFICATION FROM CULTURED CELLS

(In case of using adherent cells, trypsinize cells before harvesting)



1)

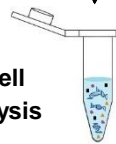
a) Transfer up to  $1 \times 10^7$  cells to a 1,5-ml microcentrifuge tube, and harvest by centrifugation for 20 seconds at 6.000g. Discard the supernatant and add **150µl Red Blood Cell Lysis Buffer**. Mix by inversion

b) In case of fresh blood (except human blood), add **150µl of Buffer BC2**, and mix by shaking vigorously.

- i) For mammalian blood sample (non-nucleated), the sample can be up to 50µl
- ii) For nucleated erythrocytes (e.g. fish or birds), the sample can be up to 10µl



2) Add 200µl of **Buffer BC1**, mix by shaking vigorously for 5 seconds, and incubate at **70°C** for 10 minutes or sample lysate is clear. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70°C water bath to be used in step 9].



3) [*Optional step; If RNA-free DNA is required*] Allow the mixture to cool to room temperature and add 5µl of **RNase A (10mg/ml)**, vortex, and incubate for 5 minutes at room temperature.

4) Add 200µl of absolute ethanol to the lysate and mix immediately by shaking vigorously for 10 seconds. In case precipitate appears, break it up by pipetting.



5) Place the **genomic DNA mini spin column** in a 2-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.

6) Centrifuge at 14.000g-16.000g for 2 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.



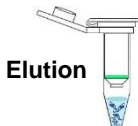
7) Add **400µl of Wash Buffer 1** and centrifuge at 14.000g-16.000g for 30 seconds. Discard the flow-through and place the genomic DNA mini spin column back in the collection tube. Add **600µl of Wash Buffer 2\*** and centrifuge at 14.000g-16.000g for 30 seconds (**\*check if ethanol is added first time the kit is used; see Notes on page 2**).

8) Discard the flow-through and place the genomic DNA mini spin column back in the collection tube and centrifuge for another 3 minutes at 14.000g-16.000g to dry the matrix of the column.



9) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 100µl **preheated Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3-5 minutes.

**Notes:** Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5. To increase DNA concentration elute with 30-50µl. To increase overall yield, elute with 200µl.



10) Centrifuge for 30 seconds at 14.000g-16.000g to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20°C.

## TROUBLESHOOTING

### 1. Low Yield

- *Clogged Column*
  - i. Reduce the amount of sample material
- *Precipitate was formed at DNA Binding Step*
  - i. Reduce the amount of sample material
  - ii. Prior to loading the column, break up precipitate in ethanol-added lysate
- *Incorrect DNA Elution Step*
  - i. Ensure that the Elution Buffer is completely adsorbed after being added to the centre of the spin column
- *Incomplete DNA Elution*
  - i. Elute twice to increase overall yield

### 2. Low Quality

- *Low performance in downstream applications*
  - i. Residual ethanol contamination interferes with downstream applications. Following the wash step, dry the spin column with additional centrifugation for 5 minutes or incubation at 60°C for 5 minutes in order to evaporate ethanol.
  - ii. DNA denaturation/fragmentation (which can be detected by gel analysis), may be the result of improper/long storage of “fresh” blood.

**ORDERING INFORMATION – GRS Nucleic Acid Purification Kits**

Reference #	Product Name	Quantity
GK01.0100	GRS PCR & Gel Band Purification Kit	100 preps
GK18.0500   GK18.2000	Exo/SAP Go PCR Purification Kit	500 rxn   2000 rxn
GK19.0005   GK19.0025   GK19.0060	Xpert Purification SPRI Magnetic Beads	5 mL   25 mL   60 mL
GK02.0100	GRS Genomic DNA Kit – Blood & Cultured Cells	100 preps
GK03.0100	GRS Genomic DNA Kit – Tissue	100 preps
GK04.0100	GRS Genomic DNA Kit – Plant	100 preps
GK05.0100	GRS Pure DNA Kit	100 preps
GK06.0100	GRS Genomic DNA Kit – BroadRange	100 preps
GK07.0100	GRS Genomic DNA Kit – Bacteria	100 preps
GK08.0100	GRS Total RNA Kit - Blood & Cultured Cells	100 preps
GK09.0100	GRS Total RNA Kit – Tissue	100 preps
GK10.0100	GRS Total RNA Kit – Plant	100 preps
GK11.0050	GRS microRNA Kit	50 preps
GK12.0050	GRS Viral DNA/RNA Purification Kit	50 preps
GK13.0100	GRS Plasmid Purification Kit	100 preps
GK15.0100	GRS Pure RNA Kit	100 preps
GK16.0100	GRS Total RNA Kit – Bacteria	100 preps
GK17.0100	GRS Total RNA Kit – Yeast & Fungus	100 preps
GK23.0100	TripleXtractor directRNA Kit	100 preps
GB23.0050   GB23.0100   GB23.0200	TripleXtractor reagent	50 mL   100 mL   200 mL
GK25.0100	GRS Genomic DNA Kit – Card	100 preps
GK26.0050	GRS FullSample Purification Kit	50 preps

**Note:** Individual components (buffers, columns, tubes, enzymes) can be purchased separately. For more information, please contact us via [info@grisp.pt](mailto:info@grisp.pt)

**GRiSP Research Solutions**

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