GRS Genomic DNA Kit – BroadRange – #GK06.0100

(FOR RESEARCH ONLY)

Sample : up to 200µl of whole blood (fresh or frozen), up to 25 mg of tissue, up to 25mg of paraffin-embedded tissue (FFPE), up to 15ml of amniotic fluid, buccal swab.
Expected Yield : 5 µg DNA (in case of whole fresh blood)
Format : spin column
Operation Time : depending on the type of sample
Elution Volume : 30-200 µl

Product Description

The GRS Genomic DNA Kit – BroadRange - provides an efficient and fast method for the purification of high quality total DNA (including genomic DNA, mitochondrial DNA and viral DNA) from a broad range of samples, including whole and frozen blood, amniotic fluid, buccal swab, tissue and FFPE. Eluted purified DNA (approximately 20-30kb) is suitable for PCR, and other enzymatic reactions.

Principle

The GRS Genomic DNA Kit – BroadRange - uses Proteinase K and 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, 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 30 minutes without phenol/chloroform extraction or alcohol precipitation, with typical DNA yields of 5 µg from 200µl of whole human fresh blood.

Quality Control

The quality of the GRS Genomic DNA Kit – BroadRange - 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 1.8-2.0) and further checked by agarose gel electrophoresis.

Caution

Buffer BR2 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)

Buffer BR1	30 ml
Buffer BR2	40 ml
Wash Buffer 1	45 ml
Wash Buffer 2*	25 ml
Elution Buffer	30 ml
RNase A (10mg/ml)	0,55 ml
Proteinase K **	2x11mg
gDNA plus 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
15-ml centrifuge tubes
Xylene
micropestles
PBS[1]

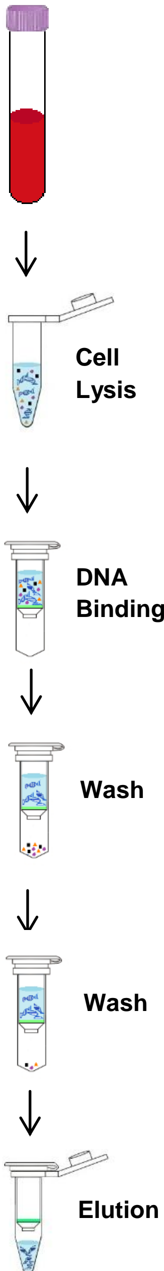
Notes

* 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 1,1 ml of ultrapure water to each vial containing 11 mg of Proteinase K to obtain a solution with a final concentration of 10mg/ml. Vortex and store at 4ºC.
[1]PBS (Phosphate Buffered Saline) = 137 mM NaCl; 2.7 mM KCl; 10 mM Na_2HPO_4; 2.0 mM KH_2PO_4, pH 7.4

Storage

RNase A (10mg/ml) should be stored at -20ºC. 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 37ºC followed by cooling to 25ºC.

PROTOCOL FOR DNA PURIFICATION FROM WHOLE BLOOD



1) Transfer 50 to 200µl of whole blood (from a blood collection tube containing EDTA or other anticoagulant) to a 1,5-ml microcentrifuge tube. Adjust volume to 200µl with PBS, and add **20µl** of **Proteinase K (10mg/ml)** to the sample. Mix by pipetting. Incubate at **60ºC** for **5 minutes.**

2) Add **200µl** of **Buffer BR2** and mix by shaking vigorously. Incubate at **60ºC** for **5 minutes**. During incubation invert the tube regularly. [At this time, preheat the Elution Buffer in a 60ºC 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 by shaking vigorously immediately for 10 seconds. In case precipitate appears, break it up by pipetting.

5) Place the **gDNA plus spin column** in a 2,0-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.

6) Centrifuge at 14.000g-16.000g for 1 minute. If the mixture did not flow-through the membrane completely, increase centrifugation time. Discard the collection tube containing the flow-through and place the 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 gDNA plus 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 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 (in case of amniotic fluid protocol: incubate at 37ºC for 5-10 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.

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 -20ºC.

PROTOCOL FOR DNA PURIFICATION FROM PARAFIN-EMBEDDED TISSUE

1) Slice up to 25 mg from blocks of FFPE tissue and transfer to a 1,5-ml microcentrifuge tube.
2) Add **1 ml of Xylene** and mix by shaking vigorously
3) Incubate at **room temperature** for **10 minutes.** During incubation, invert tube regularly. Centrifuge at 14.000g-16.000g for 3 minutes. Discard supernatant.
4) Add **1 ml** of **absolute ethanol** and mix by inverting ten times. Centrifuge at 14.000g-16.000g for 3 minutes. Discard supernatant.
5) Repeat step 4. After removing the supernatant, open the tube and incubate at 37ºC for 15-20 minutes to evaporate any remaining ethanol.
6) Proceed with **step 2** of the **Tissue protocol (see hereunder)**

PROTOCOL FOR DNA PURIFICATION FROM TISSUE

1) Cup to 25 mg of animal tissue (or 5 mm of mouse tail) and transfer to a 1,5-ml microcentrifuge tube. In case of some tissues, like spleen that contain a very high number of cells, the starting material should be reduced to 10 mg. Using a micropestle to grind the tissue to pulp will increase yield.
2) Add **200µl of Buffer BR1** and **20µl of Proteinase K** (10mg/ml). Mix by vortexing. Incubate at **60ºC overnight** (or until lysate becomes clear). Occasional or continues shaking is recommended.
3) Add **200µl of Buffer BR2**. Mix by shaking vigorously. If insoluble material is present following incubation, centrifuge at 14.000g-16.000g for 2 minutes and transfer the supernatant to a new 1,5-ml microcentrifuge tube [At this time, preheat the Elution Buffer in a 60ºC water bath].
4) Continue with **step 3** on **page 3**

PROTOCOL FOR DNA PURIFICATION FROM BUCCAL SWAB

1) Add **500µl of Buffer BR1** and **20µl of Proteinase K** (10mg/ml) to a 1,5-ml microcentrifuge tube, place the swab into the tube and incubate at **60ºC** for **10 min.**

2) Discard the swab and add **500µl of Buffer BR2**. Immediately, mix by shaking vigorously and then incubate at **60ºC** for another **10 min**. [At this time, preheat the Elution Buffer in a 60ºC water bath to be used later].

3) Add **500µl of absolute ethanol** to the lysate and mix immediately for 10 seconds by shaking vigorously. In case precipitate appears, break it up by pipetting.

4) Place the **gDNA plus spin column** in a 2,0-ml collection tube and transfer 700µl of the sample mixture (including any precipitate if present) to the column.

5) Centrifuge at 14.000g-16.000g for 1 minute. Discard the flow-through and repeat the binding step with the remaining mixture from step 3. After centrifugation, discard the collection tube containing the flow-through and place the column in a new 2,0-ml collection tube. Proceed with the wash step (**step 7 on page 3**).

PROTOCOL FOR DNA PURIFICATION FROM AMNIOTIC FLUID

1) Transfer approximately 10ml of amniotic fluid to a 15-ml centrifuge tube and centrifuge at 14.000g-16.000g for 3 minutes. Discard the supernatant

2) Resuspend the pellet with **200µl** of **Buffer BR1** and transfer the suspension to a 1,5-ml microcentrifuge tube.

3) Add **10µl of Proteinase K** (10mg/ml) and shake vigorously. Incubate at 60ºC for 30 minutes. During incubation, invert the tube regularly.

4) Add **200µl of Buffer BR2** and mix by shaking vigorously. Incubate at 60ºC for 20 minutes or until lysate is clear. During incubation, invert the tube regularly [At this time, pre-heat the Elution Buffer to 60ºC].

5) Proceed with **step 3** on **page 3**.

NOTES:

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 60ºC 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 (kit)
GK01.0100	GRS PCR & Gel Band Purification Kit	100 preps
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
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

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