GRS FullSample Purification Kit
GK26.0050 (50 preps)

(FOR RESEARCH ONLY)

Sample :	up to 200 µl of biological fluids, 500 µl of whole blood, 25 mg of animal tissue, or $5x10^6$ cultured animal cells.
Expected Yield :	typically up to 9 µg of pure genomic DNA, 20 µg of total RNA, and 120 µg of protein (from $1.5x10^6$ HeLa cells)
Format :	spin column
Operation Time :	25 minutes for DNA & RNA, approximately 1 hour for protein precipitation
Elution Volume :	30-200 µl for DNA, 25-50 µl for RNA, protein resuspension in desired volume.

Product Description

The GRS FullSample Purification Kit provides an efficient and fast method for the simultaneous purification of genomic DNA, total RNA (including miRNA), and total protein from whole blood and other biological fluids, animal tissues, and cultured cells.

Principle

After lysis, DNA is first bound to a DNA binding spin column and the flow-through is subsequently passed through a RNA binding spin column. The proteins in the second flow-through can then be precipitated with acetone. Contaminants are washed and DNA is eluted from the DNA binding spin column with a low salt buffer, whilst RNA is eluted from the RNA binding spin column with RNase-free water. The entire procedure can be completed in less than half an hour without phenol/chloroform or alcohol precipitation. Eluted gDNA (20-30 kb) is suitable for most common downstream applications, including PCR, whereas purified total RNA (including microRNA) is ready to use for RT-PCR, primer extension, and Northern Blotting. After protein precipitation, the pellet is washed with ice-cold ethanol and resuspended in an appropriate volume of desired buffer compatible with the downstream application of choice.

Quality Control

The quality of the GRS FullSample Purification Kit is tested on a lot-to-lot basis by isolating genomic DNA, total RNA, and total protein from cultured animal cells. Quantity and Purity of DNA and RNA is determined by spectrophotometer and verified by electrophoresis on a 1% agarose gel. Protein is quantified by Bradford Assay and analyzed on SDS-PAGE.

Caution

Some buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles. In order to prevent RNase contamination, one should use disposable plastic ware. Automatic pipettes and non-disposable glassware or plasticware should be sterile/RNase-free and used only for RNA procedures. During handling, gloves should be worn at all times.

Kit Contents (50 preps)

Red Blood Cell Lysis Buffer	100 ml
DRP Lysis Buffer	30 ml
DNA Wash Buffer 1	45 ml
DNA Wash Buffer 2*	12.5ml
Wash Buffer RNA 1	30 ml
Wash Buffer RNA 2**	25 ml
DNA Elution Buffer	30 ml
RNase-free Water	2x6ml
8M Urea	6 ml
Genomic DNA mini spin column	50
RNA mini spin column	50
2,0-ml collection tube	200
1,5-ml microtube (DNase/RNase-free)	150
DNase I solution**	275 ul
DNase I reaction buffer **	2,5 ml

Required Components (not included)

Ethanol (96%-100%)
ß-mercaptoethanol
PBS pH 7.2 (phosphate buffered saline)
Acetone
Trypsin (0.10-0.25%) or Accutase®
TissueLyser or mortar and pestle
20-G needle syringe
Ice
Waterbath
15-ml centrifuge tubes
2-ml centrifuge tubes
Ceramic or stainless steel beads

Notes

*Add 50 ml ethanol (96%-100%) [not included] to DNA Wash Buffer 2 prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed.
**Add 100 ml ethanol (96%-100%) [not included] to Wash Buffer RNA 2 prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed.
*** DNase I solution and reaction buffer are shipped at room temperature and should be stored at -20ºC for up to 1 year. One should consider to prepare small aliquots, as it is recommended not to repeat thawing and freezing more than 3 times.

Storage

DNase I, and DNase I reaction buffer 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. Close bottle containing DNA Wash Buffer 2 and Wash Buffer RNA 2 tightly to avoid ethanol evaporation.

DNase I treatment of RNA

DNA contamination in the final RNA solution interferes with several downstream applications, such as gene expression analysis. The amount of DNA contamination in the RNA eluate can be significantly reduced by DNase I treatment of the sample. This can be conveniently done “in column” (see step 11 of the RNA purification protocol on page 6 . We highly recommend to use the reaction buffer included in this kit as standard DNase buffers often are incompatible with in column DNase I treatment and might compromise RNA yield and integrity. For some very sensitive applications it might be necessary to eliminate even the smallest amounts of residual DNA. In order to effectively remove any trace amounts of DNA, one should consider to treat the eluted RNA with DNAse I as described hereunder.

DNA Digestion in Solution (Optional)

Mix as follows in a RNase-free microtube:

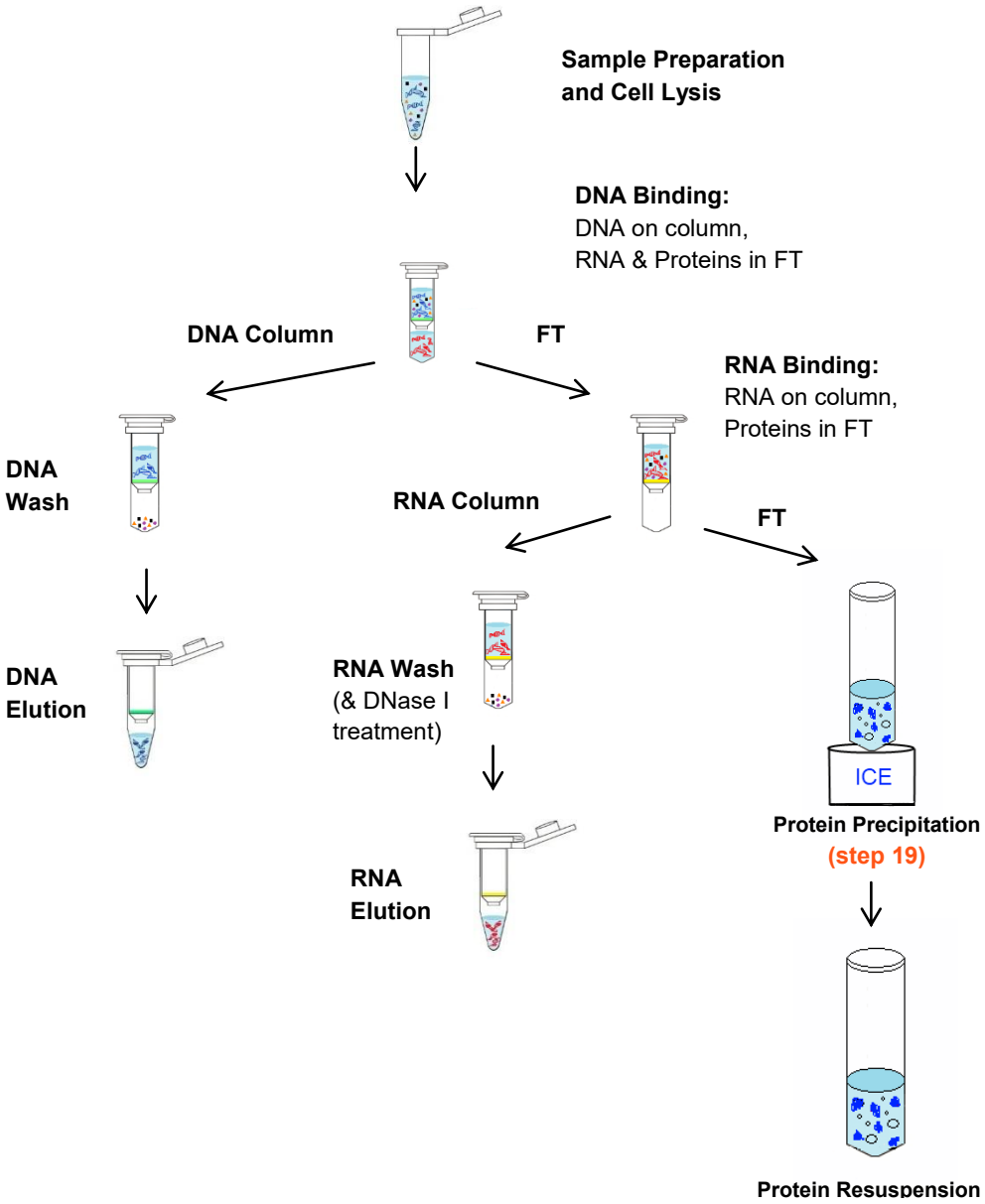
- Purified RNA (in RNase-free water): 5-40 µl
- DNase I Reaction Buffer (1x): 5 µl
- DNase I Solution : 0,5 µl for each µg of purified RNA
- RNase-free water: make up to final volume of 50 µl

Incubate at 37ºC for 15-30 minutes and stop the reaction by adding 1µl of 20mM EGTA (pH 8.0) and heating at 65ºC for 10 minutes.

DNase I can be removed from the reaction mixture by standard phenol extraction. Alternatively, add 250 µl of buffer R1 and 300µl of 70% ethanol (prepared with RNase-free water) and mix well by vortexing. Transfer all of the mixture to a new RNA mini spin column and centrifuge at 14.000-16.000g for 1 minute. Discard the flow-through and proceed with step 9 on page 3. Note that following this option the total amount of RNA purifications that can be done with this kit will be reduced.

Work-Flow and Time Consideration

DNA, RNA, and Proteins are purified sequentially from the same sample. First DNA is bound to the genomic DNA mini spin column and the flow-through (FT) is then being used for subsequent RNA and Protein Purification. Before continuing with the DNA purification, one could consider to store the genomic DNA mini spin column in a new collection tube at room temperature or at +4ºC until reaching step 19 (see work-flow below), which includes a 30 minutes waiting step. The flow-through, containing RNA and proteins is then passed through a RNA binding column. RNA binds to the column, whereas proteins do not. Once again, once could consider to continue with RNA purification, once reached step 19 (protein precipitation), in order to save a considerable amount of time on the overall procedure.



Sample Preparation and Cell Lysis

Sample preparation should be done at room temperature. Quality and Yield of DNA and RNA will be higher with fresh samples or samples which have been frozen quickly and stored at -70ºC. Freeze-thaw cycles may degrade DNA and/or RNA.

a. Tissues

Add 10-25 mg tissue sample in a 2-ml centrifuge tube (not supplied) containing ceramic or stainless steel beads (not supplied) and add **400 µl of DRP Lysis Buffer** and **4 µl of ß-mercaptoethanol** (not supplied). Homogenize sample using a TissueLyser, cell disrupter, or similar. **Alternatively,** freeze the tissue in liquid nitrogen and grind thoroughly with a tissue grinder or mortar. Transfer the tissue powder to a 1.5ml-microtube (do not allow the tissue to thaw) and add **400 µl of DRP Lysis Buffer** and **4 µl of ß-mercaptoethanol** (not supplied). Shear the tissue by passing the lysate 10 times through a 20-G needle. Proceed with step 1 of the DNA/RNA/Protein purification.

b. Adherent Cells

Remove the culture medium and wash the cells in PBS. Aspirate off PBS and trypsinize with 0.10-0.25% trypsin (not supplied) in PBS or detach cells using Accutase®. Once cells are detached, add fresh culture medium and continue with step c) Cells in Suspension (see below).

c. Cells in Suspension

Transfer up to $5x10^6$ cells to a 1.5-ml RNase-free microtube, harvest by centrifugation (5 min at 3000g) and discard supernatant. Lyse cells in **400 µl of DRP Lysis Buffer** supplemented with **4 µl of ß-mercaptoethanol** (not supplied) by pipetting up and down several times. Proceed with step 1 of the DNA/RNA/Protein purification.

d. Body Fluids

Transfer up to 200 µl to a 1.5-ml RNase-free microtube and add 3 volumes of **DRP Lysis Buffer** supplemented with **ß-mercaptoethanol** (100:1) (e.g. 100µl body fluids with 300 µl Buffer FS1 and 3 µl ß-mercaptoethanol). Mix well by vortexing. Proceed with step 1 of the DNA/RNA/Protein purification.

e. Whole blood (human)

Transfer up to 500 µl of anticoagulant-treated fresh blood to a 15-ml centrifuge tube (not supplied). Add 3 volumes of Red Blood Cell Lysis Buffer and mix by inversion. Incubate on ice for 10 minutes and vortex briefly once or twice during incubation. Centrifuge at 3000g for 5 minutes and discard the supernatant. Add **400 µl of DRP Lysis Buffer** supplemented with **4 µl of ß-mercaptoethanol** (not supplied) and resuspend the leukocyte pellet by pipetting up and down several times. Proceed with step 1 of the DNA/RNA/Protein purification.

2. DNA/RNA/Protein Purification

2.1. DNA Binding and Purification

1. Incubate the sample lysate at room temperature for 5 min. Centrifuge at 14.000g-16.000g for 2 minutes. Meanwhile, place a Genomic DNA mini spin column in a 2,0-ml collection tube.

2. Transfer the supernatant to the column. Centrifuge at 14.000g-16.000g for 90 seconds, or until the whole supernatant has passed through the column. Place the Genomic DNA mini spin column in a new 2-ml collection tube and **Save** the flow-through as it contains RNA and Proteins, for **step 8**. Continue with DNA Purification, or store the Genomic DNA mini spin column at room temperature for short period or at +4ºC for longer period. Do not freeze the column.

3. Add **400 µl of DNA Wash Buffer #1** to the Genomic DNA mini spin column. Centrifuge for 30 seconds at 14.000g.16.000g and discard the flow-through. Place the spin column back in the collection tube. Add **600 µl of DNA Wash Buffer #2** (verify that ethanol was added). Centrifuge at 14.000g-16.000g for 30 seconds and discard the flow-through.

4. Place the spin column back in the 2-ml collection tube and centrifuge for 3 minutes at 14.000g-16.000g to dry the column membrane completely and to get rid of trace amounts of ethanol. Discard flow-through and collection tube.

5. Remove the spin column carefully and place into a new 1.5-ml microtube.

6. Pipet **100 µl DNA Elution Buffer (pre-heated at 60ºC)** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3 min.
   Notes: Instead of DNA Elution Buffer, DNA can also be diluted with TE or water; pH should be 8.0-8.5. Standard elution volume is 100 µl. Concentration can be increased by using less volume (30 µl-50 µl) or alternatively yield can be increased by using more volume (200 µl).

7. 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 -20ºC.

2.2 RNA Binding and Purification

8. Add 0.8 volumes of 100% ethanol to the flow-through of step 2 and mix well.

9. Transfer the mixture to a RNA mini spin column (individually packed and already placed in a 2-ml collection tube)

10. Centrifuge at 14.000g.16.000g for 60 seconds. Place the RNA mini spin column in a new 2-ml collection tube and **Save** the flow-through as it contains Proteins, for **step 18**. (If the lysate did not pass completely through the column, increase centrifuge time). Continue with RNA Purification immediately.

11. [*optional (see page 2)*] Add **400 µl** of **Wash Buffer RNA #2** and centrifuge at **14.000g-16.000g** for **30 seconds**. Discard the flow-through and place the RNA mini spin column back in the collection tube. Mix for each prep **45 µl** of **DNase I reaction buffer** with **5 µl** of **DNAse I solution** in a RNase-free tube, and then pipet **50 µl** to the centre of each spin column. Incubate at **room temperature** for **10-15 minutes**. (2000 KU/ml) mixed in reaction buffer (see page 2) to the centre of the spin column and incubate at room temperature for 10 minutes.

12. Add **400 µl of Wash Buffer RNA #1** and centrifuge at 14.000g-16.000g for 1 minute. Discard the flow-through and place the RNA mini spin column back in the collection tube.

13. Add **600 µl of Wash Buffer RNA #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).**

14. Discard the flow-through and and place the RNA mini spin column back in the collection tube, and add **600 µl of Wash Buffer RNA 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).**

15. Discard the flow-through and place the RNA 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.

16. Transfer the spin column to a new 1,5-ml microcentrifuge tube (RNase-free) and pipet 25-50 µl **RNase-free Water** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3 minutes.

17. Centrifuge for 1 minute at 14.000g-16.000g to elute purified total RNA. Discard the spin column and use RNA immediately or store at -20ºC.

2.3. Protein Precipitation and Reconstitution

18. Transfer the flow-through of step 10 to a 15-ml centrifuge tube (not supplied) and add 4 volumes of ice-cold acetone (not included).

19. Incubate on ice or at -20ºC for 30 minutes.

20. Centrifuge at 14.000g-16.000g for 10 minutes and discard the supernatant.

21. Add 100 µl of ice-cold 70% ethanol to wash the protein pellet. Centrifuge at 14.000g-16.000g for 1 minute and discard the supernatant. Air-dry the pellet at room temperature.

22. Add up 100 µl 8M urea (included) or a buffer of choice, compatible with desired down-stream application.

TROUBLESHOOTING

1. **Low DNA and/or RNA Yields**
   - *Sample preparation*
     i. Yield and quality of the purified DNA and RNA depend on the quality of the sample and will be higher in case of fresh samples than in case of frozen (repeatedly) samples. Increased storage time decreases yield. Too much sample, thus overloading the columns, causes low nucleic acid yields.
     ii. Verify that absolute ethanol was added to the DNA Wash Buffer 2 and Wash Buffer RNA 2. Be sure and close bottles tightly each time to avoid ethanol evaporation.
   - *Incomplete DNA or RNA elution*
     i. If using water to elute the DNA, make sure that the pH is above 7,5. Water should be prepared freshly as CO_2 from the environment could cause acidification and lower the DNA´s solubility. Preheat Elution Buffer to 60ºC to increase yield.
     ii. Ensure that Elution Buffer, or RNase-free water is added to the Centre of the column and is completely adsorbed. Elute twice (second time with eluate) to increase 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

3. **Low Protein**
   - *No protein detected by SDS-PAGE or Western Blotting*
     i. After protein precipitation, pellet is only loosely attached to the centrifuge tube. Decant supernatant carefully