GRS circulating cell-free DNA/RNA Purification Kit
#GK20.0050 | 50 preps

(FOR RESEARCH ONLY)

Sample :	1-5 ml of serum or plasma
Expected Yield :	1-100 ng of DNA/RNA per ml of serum or plasma
Format :	spin column
Operation Time :	60 minutes
Elution Volume :	30-50 µl

Product Description

The GRS circulating cell-free DNA/RNA Purification Kit provides an efficient and fast method for the isolation of high-quality DNA and RNA from up to 5 ml of serum or plasma. The purified DNA/RNA is suitable for a wide variety of downstream applications including qPCR and DNA sequencing.

Principle

The GRS circulating cell-free DNA/RNA Purification Kit includes a specially developed removable extension tube that, when inserted in the mini spin column, allows for the expansion of the starting volume up to 5 ml. Antibodies and other proteins are eliminated by Proteinase K digestion, and the digest is mixed with a unique Binding Buffer that is optimized to allow easy binding of DNA and RNA to the glass fiber matrix of the spin column. The mixture can be passed through the column either by centrifugation or by using a vacuum manifold system. Contaminants are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified DNA/RNA is subsequently eluted with RNase-free water. The entire procedure can be completed in approximately 60 minutes.

Quality Control

The quality of the GRS circulating cell-free DNA/RNA Purification Kit is tested on a lot-to-lot basis by purifying DNA/RNA from 1 ml of plasma, followed by qPCR for analysis of the integrity.

Caution

The Binding Buffer contains chaotropic agents. 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)
Buffer CCF-1	220 ml
Buffer CCF-2*	330 ml
Elution Buffer	1 ml
Wash Buffer 1	50 ml
Wash Buffer 2**	12.5 ml
Carrier RNA***	1 mg
Proteinase K****	135 mg
CFF mini spin column	50
CFF column extension tube	50
2-ml collection tube	50
1.5-ml microtube (DNase/RNase-free)	50

Required Components (not included)
Isopropanol (2-propanol)
Ethanol (96%-100%)
50-ml centrifuge tubes
Centrifuge for microtubes
Pipettes (and tips)
Vortex
Water bath or Thermoblock
(optional) Vacuum manifold system or
Centrifuge for 50-ml centrifuge tubes
Ice
(optional) DNase I and/or RNase A

Notes

* Add 200 ml isopropanol [not included] to Buffer CCF-2 prior to initial use. After isopropanol has been added, mark the bottle to indicate that this step has been completed.

** Add 50 ml ethanol (96%-100%) [not included] to the 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 ml of RNase/DNase-free ddH_2O to the Carrier RNA. Vortex briefly in order to ensure that it has been completely dissolved to obtain a working solution of 1µg/µl. Centrifuge briefly and prepare aliquots, using RNase/DNase-free microtubes, of convenient volume (e.g. of 100µl), as Carrier RNA should be stored at -20ºC and repeated freeze/thaw cycles should be avoided (not more than 3 times).

*** Add RNase/DNase-free ddH_2O to the Proteinase K as indicated on the bottle(s) and store at -20ºC.

Storage

Carrier RNA should be stored at -20ºC. Proteinase K retains enzymatic activity at room temperature for extended periods, however, it is recommended to store at +4ºC for short periods and at -20ºC for prolonged periods. All other components should be stored at room temperature. Carrier RNA should be Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37ºC followed by cooling to 25ºC.

BEFORE STARTING

1. **Verify that isopropanol has been added to Buffer CCF-2, ethanol to Wash Buffer 2 and water to Carrier RNA and Proteinase K, as described in the notes on page 2.**

2. Pre-heat water bath to 60ºC

3. For each sample, pre-mix Buffer 0.8 volumes of Buffer CCF-1 and 1µl Carrier RNA (see table). **At this stage DO NOT mix with serum or plasma!**

For purifying sample vol. (ml)	Buffer CCF-1 (ml)	Carrier RNA (µl)
1	0.8	1
2	1.6	1
3	2.4	1
4	3.2	1
5	4.0	1

SAMPLE PREPARATION

4. Add, **in this particular order**, Proteinase K, sample (serum or plasma), and Buffer CCF-1 (already containing Carrier RNA (step3)), to a 50-ml DNase/RNase-free centrifuge tube, in the amounts set in the table below. Close the cap and vortex for 30-60 seconds.

For purifying sample vol. (ml)	Proteinase K (µl)	serum/plasma (ml)	Buffer CCF-1 with RNA (ml)
1	100	1	0.8
2	200	2	1.6
3	300	3	2.4
4	400	4	3.2
5	500	5	4.0

5. Incubate at 60ºC for 30 minutes.

6. Add 1 volume of Buffer CCF-2 directly to 1 volume of sample mixture (see table below), vortex for 10 seconds and put on ice for 5 minutes.

For purifying sample vol. (ml)	sample mixture after Incubation (ml)	Buffer CCF-2 (ml)
1	1.9	1.9
2	3.8	3.8
3	5.7	5.7
4	7.6	7.6
5	9.5	9.5

Column loading (using centrifuge)

7. The CCF extension tube has already been inserted in a CCF mini spin column, which in turn is inserted in a 2-ml collection tube. Remove the 2-ml collection tube and keep for later use. Verify that the CCF extension tube maintains well inserted in the CCF mini spin column.

8. Press the lid of the CCF mini spin column down and slide the combination into a clean 50-ml centrifuge tube.

9. Transfer 10ml of the sample mixture to the assembly and centrifuge at 1,500g for 2 minutes. Discard the flow-through, and repeat this step until the entire sample mixture has been passed through the column. After final passage, disconnect the CCF mini spin column and place in the 2-ml collection tube. Continue with step 13.

Column loading (using a vacuum manifold system)

10. The CCF extension tube has already been inserted in a CCF mini spin column, which in turn is inserted in a 2-ml collection tube. Remove the 2-ml collection tube and keep for later use. Verify that the CCF extension tube maintains well inserted in the CCF mini spin column.

11. Connect the assembly to a vacuum manifold system.

12. Pass the entire sample mixture through the assembly applying vacuum at 15 inches Hg (~380mmHg). Switch of the vacuum and disconnect the CCF mini spin column and place in the 2-ml collection tube. Continue with step 13.

Wash

13. Add 400 µl of Wash Buffer 1 to the centre of the CCF mini spin column and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the CCF mini spin column back in the collection tube.

14. Add 600 µl of Wash Buffer 2* to the centre of the CCF mini spin column and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the CCF mini spin column back in the collection tube. Centrifuge for an additional 3 minutes to dry the column matrix completely. (*ensure ethanol has been added).

Elution

15. Transfer the CCF mini spin column to a new 1.5-ml microcentrifuge tube (RNase/DNase-Free) and pipette 30µl-50µl* of RNase-free water directly to the centre of the spin column without touching the membrane (*30µl for higher concentration or 50µl for higher yield). Incubate at room temperature for 2-3 minutes.

16. Centrifuge for 2 minutes at 14,000g-16,000g to elute purified DNA/RNA. Discard the spin column and use DNA/RNA immediately or store at -70ºC (in case of RNA or mixture) or at -20ºC (DNA only).

Optional

Presence of DNA in the final RNA solution might interfere with some 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. We highly recommend to use GRiSP's DNase I set (cat#: GKC01.0050), which can be purchased separately, using the following protocol:

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 stop solution (20mM EGTA (pH 8.0)) and heating at 65ºC for 10 minutes.

TROUBLESHOOTING

1. **Yield**
   - *Primary Blood Collection Tube*
     - i. If the primary blood collection tube contained an anticoagulant other than EDTA, yield may be compromised as some anticoagulants accelerate DNA and RNA degradation.
   - *Incorrect sample preservation*
     - i. If plasma/serum was prepared from blood sample after an extended time, blood cells might have disintegrated and released nucleic acids into the plasma, compromising the target nucleic acids. In addition, repeated freezing and thawing may lead to DNA/RNA degradation.
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. Carrier RNA in the eluate may interfere with some downstream applications. If this is the case, reduce the amount of carrier RNA or omit it completely from the procedure.

ORDERING INFORMATION – GRS Nucleic Acid Purification Kits

Reference #	Product Name	Quan tity (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
GK20.0050	GRS circulating cell-free DNA/RNA Purification Kit	50 preps
GK21.0100	GRS Genomic DNA Kit – Food	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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