GRISP
RESEARCH SOLUTIONS

GRS Circulating Cell-Free DNA/RNA Purification Kit

#GK20.0050 (50 preps)
(FOR RESEARCH ONLY)

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

Product: 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.

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.

QC: 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 salt which is a harmful irritant. 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****	300 ml
Wash Buffer 1	50 ml
Wash Buffer 2*	12.5 ml
RNase-free Water	30ml+6ml
Carrier RNA***	1mg
Proteinase K**	5x 55mg
CCF mini spin column e extension tube	50 each
1.5-ml microtube (DNase/RNase free)	50
2-ml collection tube	50

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex
Water bath or Thermoblock
Single hole paper punch
Isopropanol (2-propanol)
50-ml centrifuge tubes
Ice
DNase I

* Add Ethanol (96%-100%) [not included] to Wash Buffer 2, as indicated on the bottle/tube, prior to initial use. After Ethanol has been added, mark the bottle/tube to indicate that this step has been completed. Close bottle tightly to avoid ethanol evaporation.

**Add Water (ultrapure) [not included] to Proteinase K, as indicated on the tube, prior to initial use.
After Water has been added, mark the tube to indicate that this step has been completed.

***Add 1 ml of Elution Buffer to the Carrier RNA, as indicated on the bottle/tube, prior to initial use.
After Elution Buffer has been added, vortex and ensure that RNA is completely dissolved. Aliquot the Carrier RNA in convenient amounts in RNase-free microtubes and store at -20ºC. Avoid freeze-thawing for more than 3 times.

**** Add 200ml isopropanol to Buffer CCF-2, as indicated on the bottle, prior to initial use. After isopropanol has been added, mark the bottle/tube to indicate that this step has been completed.

Storage: Store Carrier RNA at -20ºC. Proteinase K is a stable enzyme and transport is carried out either with or without cooling. Upon arrival, proteinase K (powder) should be stored at 4ºC. Once water has been added, it is recommended to store the Proteinase K solution 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. Store for up to 2 years.

PRIOR TO USE

Ensure ethanol was added to Wash Buffer 2, water to Proteinase K and to Carrier RNA, and isopropanol to Buffer CCF-2. Then, for each sample to be processed, mix 1µl of RNA Carrier Solution and 0.8 volumes of Buffer CCF-1 (according to table). At this stage: **DO NOT Mix with Serum or Plasma**. Meanwhile, preheat water batch to 60ºC.

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

PROTOCOL

1. Add, in this particular order: Proteinase K, sample (serum or plasma) and Buffer CCF-1 (already containing Carrier RNA (see prior to use), 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 carrier 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

2. Incubate at 60ºC for 30 minutes.

3. 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 Mix after step 2 (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

4. 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.

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

6. 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.

7. Add 400 µl of Wash Buffer 1 to the center 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.

8. Add 600 µl of Wash Buffer 2* to the center 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).

9. 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 center 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.

10. 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).

USING A VACUUM MANIFOLD SYSTEM

Instead of centrifugation, one can use a vacuum manifold system for column loading, washing and elution. For this, replace step 5 and 6 on page 3 by the following step 5 and 6 and continue afterwards with step 7 as described above.

5. Connect the assembly to a vacuum manifold system.

6. 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.

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#: GKC01.0100), 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. **Low 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. Verify that Carrier RNA was added to Buffer C1.
   - *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.