GRISP
RESEARCH SOLUTIONS

GRS Genomic DNA Kit – Food

#GK21.0100 (100 preps) | GK21s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample :	200mg of raw or processed food samples
Expected Yield :	up to 50µg of DNA
Format :	spin column
Operation Time :	within 60 minutes
Elution Volume :	30-200µl

Product: The GRS Genomic DNA Kit – Food – provides an efficient and fast method for the purification of high-quality DNA from a broad variety of raw and processed food samples, suitable for all common downstream applications such as PCR, enzymatic restriction digestion, cloning, Southern blot analysis, etc.

The GRS Genomic DNA Kit – Food – can be used for both raw and processed food samples. When combined with Proteinase K, the specific lysis buffer (containing CTAB), results in the digestion of food tissues and proteins. Common PCR inhibitors (polysaccharides and plant metabolites) are removed by centrifugation whereas residual inhibitors are separated by chloroform (not included). The cleared supernatant is subsequently mixed with a binding buffer that is optimized to allow selective binding of DNA to the glass fiber matrix of the spin column. Remaining contaminants are completely removed using Wash Buffer. The purified genomic DNA is subsequently eluted by a low salt Elution Buffer or TE or water. The entire procedure can be completed in less than 60 minutes without phenol/chloroform extraction or alcohol precipitation.

QC: The quality of the GRS Genomic DNA Kit – Food – is tested on a lot-to-lot basis by isolating genomic DNA from 200mg food samples. The purified DNA (A260/A280 ratio of 1.8-2.0) is analyzed by electrophoresis.

Caution: During operation, always wear a lab coat, disposable gloves, and protective goggles. Chloroform (required but not included in this kit) is a hazardous chemical. Due to its volatility it should be handled either in a glove box or in a well-ventilated fume hood. We highly recommend the manufacturer´s instructions and material safety data sheet. Chloroform has to be disposed as hazardous waste in an appropriate container, according to national and local regulations.

Kit Contents	(100 preps)	(4 preps)
Food Lysis Buffer	200 ml	10 ml
Binding Buffer	100 ml	4 ml
Wash Buffer 1	45 ml	2 ml
Wash Buffer 2*	25 ml	1 ml
Elution Buffer	30 ml	1 ml
Proteinase K**	11 mg	1 mg
Genomic DNA mini spin column	100	4
1.5-ml microtube (DNase/RNase free)	300	-
2-ml collection tube	100	4

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex
Water bath or Thermoblock
2-ml centrifuge tubes (or larger)
Chloroform
Ice
Tissue lyser/Mortar+Liquid Nitrogen+Pestle

* Add Ethanol (96%-100%) [not included] to Wash Buffer 2, as indicated on the bottle, prior to initial use. After Ethanol has been added, mark the bottle 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.

Storage: 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.

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PROTOCOL FOR DNA PURIFICATION FROM FOOD SAMPLES

1) Homogenize (up to) 200mg of food sample as usual (e.g., with stainless steel or ceramic beads using a *TissueLyser* or *Disruptor Genie* (or Similar), or by transferring to a mortar, add liquid nitrogen and grind thoroughly using a pestle). If food sample is liquid, transfer directly to a 2-ml (or larger) centrifuge tube (not included).

2) Add 1ml of Food Lysis Buffer and 10µl of Proteinase K (10mg/ml) to the microcentrifuge tube containing the homogenized food sample and vortex for 10 seconds. For samples that swell (e.g., containing starches), add another 1ml of Food Lysis Buffer to ensure that the sample is completely submerged.

3) Incubate at 60ºC for 30 minutes with constant shaking. Alternatively invert tube regularly.

4) Cool sample (on ice or in refrigerator at +4ºC) to room temperature. Centrifuge at 2,500g at room temperature for 5 minutes. Transfer 600µl of clear supernatant (maybe colored, depending on sample) to a new 1.5-ml DNase-free microcentrifuge tube (included). If material is floating on top of the supernatant, transfer only the supernatant). Make sure not to touch any pellet from the bottom of the tube.

5) Add 500µl of chloroform (not included) and then vortex for 15 seconds.

6) Centrifuge at 14,000g-16,000g at room temperature for 10 minutes. In case the supernatant is not clear yet, centrifuge for another 10 minutes. Carefully transfer 500µl of the upper aqueous phase to a new 1.5-ml DNase-free microcentrifuge tube (included).

7) Add 500µl of Binding Buffer and mix immediately. (For extraction of small DNA fragments (100-200bp) from highly processed samples: use 1ml of Binding Buffer)

8) Place the spin column back in a collection tube and transfer 700µl of the mixture to the column. Then centrifuge at 14,000g-16,000g for 1 min at room temperature.

9) Discard the flow-through, place the column back in the collection tube and transfer the remaining mixture to the column, and repeat centrifugation.

10) Discard the flow-through, place the column back in the collection tube and add 400µl of Wash Buffer 1. Centrifuge 14,000g-16,000g for 30 seconds.

11) Discard the flow-through, place the column back in the collection tube and add 600µl of Wash Buffer 2 (Ensure that ethanol was added). Centrifuge 14,000g-16,000g for 30 seconds.

12) Discard the flow-through, place the column back in the collection tube and centrifuge 14,000g-16,000g for an additional 3 minutes to dry the column matrix

13) If pigment remains on the column, wash with 500µl of absolute ethanol (in the same way as with Wash Buffer 2 in steps 11 and 12).

14) Transfer the spin column to a new 1.5-ml DNase-free microcentrifuge tube (included). and pipette 100µl of preheated (60ºC) Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for at least 2 minutes.

Notes: 1) Instead of Elution Buffer, DNA can also be diluted with TE or water; pH ideally should be 8.0-8.5. 2) Standard elution volume is 100µl. To increase concentration, elute with 30-50µl. To increase yield, elute with 200µl.

15) Centrifuge for 2 minutes at 14,000g-16,000g to elute purified DNA. Discard the spin column and use DNA immediately or store at -20ºC.

TROUBLESHOOTING

1. **Low Yield**
    - *Clogged Column*
        i. Reduce the amount of sample material
        ii. Ensure that the food material is completely homogenized
        iii. Extend incubation time to 90 minutes
    - *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 center 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.