GRS Genomic DNA Kit – Soil

#GK30.0050 (50 preps) | GK30s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample :	200mg-500mg of soil
Expected Yield :	up to 5µg DNA
Format :	bead beating combined with PCR inhibitor removal and spin column purification
Operation Time :	within 40 minutes
Elution Volume :	30-100µl

Product: The GRS Genomic DNA Kit - Soil - provides an efficient and fast method for the extraction and purification of high-quality genomic DNA from microorganisms (archaea, bacteria, fungi, and algae) found in soil samples.

Samples are disrupted and homogenized by vortexing in a lysis buffer mixed with zirconia/silica beads. Humic substances (HS), including insoluble particles, proteins and PCR inhibitors such as humic acid (HA), are precipitated using a unique buffer. Residual HA is subsequently removed using specific HA removal columns. The buffer system is optimized to allow selective binding of DNA to the glass fiber matrix of the spin column. Contaminants are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified DNA is subsequently eluted with a low salt Elution Buffer (or TE). The entire procedure can be completed within 40 minutes without phenol extraction or ethanol precipitation. Eluted purified DNA is suitable and ready-to-use for PCR, real-time PCR, Southern Blotting, RFLP and sequencing.

QC: The quality of the GRS Genomic DNA Kit - Soil - is tested on a lot-to-lot basis by isolating genomic DNA from 250mg soil samples. Quantity and Quality are ascertained by spectroscopy and gel electrophoresis.

Caution: Some buffers contain chaotropic salt, which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Kit Contents	GRS (50 preps)	sample (4 preps)
Buffer S1	50 ml	4 ml
Buffer S2	15 ml	1 ml
Buffer S3	90 ml	10 ml
Wash Buffer 2*	25 ml	1 ml
Elution Buffer	6 ml	1 ml
Bead beating tube (HS type)	50	4
HA removal column	50	4
RNase A (10mg/ml)	0.55ml	-
Genomic DNA mini spin column	50	4
2-ml centrifuge tube	50	4
1.5-ml microtube (DNase/RNase free)	100	-
2-ml collection tube	50	4

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex with adapter
Water bath or Thermoblock

* 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.

Storage: RNase A is a stable enzymes and transport is carried out either with or without cooling. Upon arrival, RNase A (10mg/ml) should be stored at -20°C. 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 37°C followed by cooling to 25°C. Store for up to 2 years.

PRIOR TO USE

Depending on the condition of the soil sample, one should consider to adapt step 1 of the standard protocol (page 3). Very dry soil can soak up large amounts of liquids and therefore it is recommended to either reduce the amount of soil or to increase the amount of Buffer S1. On the other hand, for very wet soil samples, after transferring the sample to a bead beating tube, centrifuge at 8,000g for 1 minute and remove liquid by pipetting, before adding Buffer S1. In case of frozen soil samples, it is recommended after transferring to a bead beating tube to incubate the bead beating tube at 70°C for 10 minutes, before adding Buffer S1.

All steps are at room temperature unless indicated otherwise.
Before starting, preheat the required amount of elution buffer to be used in step 13 to 60°C

PROTOCOL FOR DNA PURIFICATION FROM SOIL

1. Transfer up to 500mg of soil (*see prior to use*) to a bead beating tube (type HS), add 750µl of Buffer S1 and vortex briefly.
2. Once all samples are prepared, tape the bead beating tubes horizontally to a standard vortex or attach them using an adapter. Vortex vigorously (max speed) for 10 minutes.
3. Centrifuge at 8,000g for 2 minutes to reduce/eliminate foam that was formed during vortexing.
4. Add 150µl of Buffer S2 and mix by vortexing for 5 seconds, and incubate on ice (0°C-4°C) for 5 minutes.
5. Centrifuge at 8,000g for 2 minutes to precipitate insoluble particles and PCR inhibitors.
6. Place a HA removal column in a 2-ml centrifuge tube and transfer 500µl-600µl of the clear supernatant from the bead beating tube to the HA removal column. Avoid transferring insoluble matter.
7. Centrifuge at 14,000g-16,000g for 1 minute. Discard the HA removal column. Save the flow-through in the 2-ml centrifuge tube for subsequent DNA Binding step. If insoluble matter present in the flow-through, centrifuge briefly and transfer the clear supernatant to a new 1.5-ml microcentrifuge tube.
8. [optional; when RNA-free DNA is required] Add 10µl of RNase A (10mg/ml), vortex, and incubate at room temperature for 10 minutes.
9. Add 900µl Buffer S3 to the flow-through and mix immediately by vortexing for 5 seconds.
10. Place the Genomic DNA Mini Spin Column in a 2-ml collection tube and transfer 750µl of the sample mixture to the column. Centrifuge at 14,000g-16,000g for 2 minutes. Discard the flow-through from the collection tube and place the column back in the same collection tube. Add the remaining sample mixture from step 8 and centrifuge again for 2 minutes. Discard the flow-through from the collection tube and place the column back in the same collection tube.
11. Add 400µl of Buffer S3 to the column and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the column back in the collection tube.
12. Add 600µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds (*Ensure ethanol was added 1st time prior to use). Discard the flow-through, place the column back in the collection tube and repeat this step once.
13. Discard the flow-through, place the column back in the collection tube, and centrifuge for another 3 minutes at maximum speed to dry the matrix of the column.
14. Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipette 100µl of Elution Buffer (preheated at 60°C) directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3-5 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 at 14,000g-16,000g for 1 minute to elute purified total DNA. Discard the spin column and use DNA immediately or store at -20°C.

TROUBLESHOOTING

1. **Low Yield**
    - *Insufficient disruption and/or homogenization*
        - i. Reduce the amount of sample material, as an ample amount of space is required in the bead beating tube for efficient disruption of the sample
    - *Incomplete lysis and/or homogenization*
        - i. Reduce the amount of sample material
        - ii. Increase vortexing speed/time or use a Disrupter Genie or similar instead
    - *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. **DNA degradation**
    - *Fragmentation*
        - i. DNA denaturation/fragmentation (which can be detected by gel analysis), may be the result of too harsh mechanical disruption of the sample. Alternatively, instead of vortexing 10 minutes, after adding buffer S1, vortex at maximum speed for 5 seconds and then incubate at 70°C for 5 minutes. Repeat this vortex/incubate cycle 3 more times and continue with step 3.

3. **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 60°C for 5 minutes in order to evaporate ethanol.
        - ii. If, despite the PCR inhibitor removal process, PCR is inhibited, use a dilute (1:10) DNA sample as template or purify the genomic DNA further using the GRS Pure DNA Kit.