GRS Genomic DNA Kit – Yeast & Fungus

#GK31.0100 (100 preps) | GK31s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to $2x10^8$ cells of a large variety of yeast and fungus species
Expected Yield : ~10µg genomic DNA from $2x10^8$ cells of *S.cerevisiae*
Format : bead beating combined with spin column purification
Operation Time : within 45 minutes
Elution Volume : 30-200µl

Product: The GRS Genomic DNA Kit – Yeast & Fungus - provides an efficient and fast method for the extraction and purification of high-quality genomic DNA from a large variety of yeast and fungus cultures.

Cells are efficiently lysed by vortexing in a lysis buffer mixed with zirconia/silica beads, without the need of first forming spheroplast by Zymolyase (Lyticase) treatment. 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 45 minutes without phenol extraction or ethanol precipitation, with typical DNA yields of >8µg (for $2x10^8$ cells of *S.cerevisiae*). 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 – Yeast & Fungus - is tested on a lot-to-lot basis by isolating genomic DNA from $2x10^8$ cells of *S.cerevisiae*. 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 (100 preps)	sample (4 preps)
Buffer YF1	75 ml	3 ml
Buffer YF2	15 ml	1 ml
Buffer YF3	60 ml	2 ml
Wash Buffer 1	45 ml	2 ml
Wash Buffer 2*	25 ml	1 ml
Elution Buffer	30 ml	1 ml
RNase A (50mg/ml)	0.55ml	25µl
Genomic DNA mini spin column	100	4
Bead beating tube (YF type)	100	4
1.5-ml microtube (DNase/RNase free)	200	-
2-ml collection tube	100	4

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

*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 enzyme and transport is carried out either with or without cooling. Upon arrival, RNase A (50mg/ml) 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. Store for up to 2 years.

PRIOR TO USE

All steps are at room temperature unless indicated otherwise.
Before starting, preheat the required amount of elution buffer to be used in step 11, on page 2, to 70ºC

GRISP RESEARCH SOLUTIONS

PROTOCOL FOR DNA PURIFICATION FROM YEAST OR FUNGUS SPECIES

1. **a) From Plates:** Using an inoculation loop, transfer 50-200mg of yeast or fungus colonies (up to $2x10^8$ cells) from a culture medium plate to a 1.5-ml microtube (DNase/RNase-free).
   b) From Broth: Transfer yeast or fungus cells from liquid culture medium to a 1.5-ml microtube (DNase/RNase-free) and centrifuge at 5,000g for 10 minutes. Discard the supernatant. Weigh 50-200mg (up to $2x10^8$ cells) of wet pellet. If cell density is too low, transfer additional culture medium to the pellet and repeat until sufficient cells are accumulated in the pellet.
2. Resuspend cells in 600µl of Buffer YF1. Add 5µl of RNase A (50mg/ml) and transfer the whole mixture to a bead beating tube (type YF).
3. Once all samples are prepared, tape the bead beating tubes horizontally to a standard vortex or attach them using an adapter (or use alternative bead beating instrument). Vortex vigorously (max speed) for 10 minutes (or follow the alternative instrument manufacturer´s instructions).
4. Incubate at 70ºC for 10 minutes. Invert during incubation the tubes regularly (e.g. every 2 minutes).
5. Cautiously open the cap and add 100µl of Buffer YF2. Vortex briefly to eliminate foam caused by detergents and incubate on ice for 5 minutes.
6. Centrifuge at 11,000g for 3 minutes and then transfer 450µl of the supernatant to a new 1.5-ml microcentrifuge tube.
7. Add 450µl Buffer YF3 and 450µl of absolute ethanol to the sample and mix immediately by vortexing for 10 seconds. (Buffer YF3 and absolute ethanol can be premixed in equal volumes on the same day and stored at room temperature. In that case, add 900µl of the combined YF/ethanol mix to the sample instead of first 450µl Buffer YF3 and then 450µl of absolute ethanol).
8. Place the Genomic DNA Mini Spin Column in a 2-ml collection tube and transfer 700µl 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.
9. Add 400µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through, and place the column back in the collection tube.
10. 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 centrifuge for another 3 minutes at maximum speed to dry the matrix of the column.
11. Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipette 100µl of Elution Buffer (preheated at 70ºC) directly to the center of the spin column without touching the membrane. Incubate at room temperature for 2-3 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.
12. Centrifuge at 14,000g-16,000g for 2 minutes to elute purified total DNA. Discard the spin column and use DNA immediately or store at -20ºC.

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. Incubate sample at 70ºC for 10 minutes to facilitate cell lysis following vortex
     - 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 YF1, vortex at maximum speed for 5 seconds and then incubate at 70ºC for 5 minutes. Repeat this vortex/incubate cycle 3 more times and continue with step 3. This method is expected to reduce DNA shearing, however, may also reduce overall DNA yield.

3. **Low Quality**
   - *Low performance in downstream applications*
     - i. Residual ethanol contamination interferes with downstream applications. Following the wash step (step 10), dry the spin column with additional centrifugation for 5 minutes.