



FavorPrep™ Fungi/ Yeast Genomic DNA Extraction Mini Kit

(For Research Use Only)

Kit Contents:

Cat. No:	FAFYG 000 (4 preps_sample)	FAFYG 001 (50 preps)	FAFYG 001-1 (100 preps)
Bead Tubes	4 pcs	50 pcs	100 pcs
FA Buffer	5 ml	60 ml	120 ml
FB Buffer	2.7 ml	32 ml	65 ml
TG1 Buffer	2 ml	27 ml	55 ml
TG2 Buffer	2 ml	15 ml	30 ml
W1 Buffer ^a (concentrate)	1.3 ml	22 ml	44 ml
Wash Buffer ^b (concentrate)	1 ml	10 ml	20 ml
Elution Buffer	0.5 ml	7 ml	15 ml
Lyticase solution	250 µl	550 µl × 5	550 µl × 10
Proteinase K ^c	1 mg	11 mg	11 mg × 2
TG Mini Column	4 pcs	50 pcs	50 pcs × 2
Collection Tube	8 pcs	100 pcs	100 pcs × 2
Elution Tube	4 pcs	50 pcs	100 pcs

Preparation of W1 Buffer and Wash Buffer by adding ethanol (96 ~ 100%) and Store at RT.			
Ethanol volume for W1 Buffer ^a	0.5 ml	8 ml	16 ml
Ethanol volume for Wash Buffer ^b	4 ml	40 ml	80 ml

Preparation of Proteinase K solution by adding ddH ₂ O and Store the solution at 4°C.			
ddH ₂ O volume for Proteinase K ^c	0.1 ml	1.1 ml	1.1 ml × 2

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1~5 × 10 ⁶
Operation time:	~ 60 minutes
Binding capacity:	60 µg/ column
Column applicability:	centrifugation and vaccum

Additional requirement to be provided by user

1. Microcentrifuge capable of speed at ~18,000 × g
2. 1.5 ml microcentrifuge tube
3. 96~100 % ethanol
4. Vortex mixer
5. Heating block or water bath

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Store the Lyticase solution **at -20°C on arrival.**
3. **Caution: Lyticase solution and FB Buffer containing 14 mM of β-mercaptoethanol is hazardous to human health. perform the procedures involving Lyticase solution and FB Buffer in a chemical fume hood.**
4. Add sterilized ddH₂O to a Proteinase K tube to make a **10 mg/ml** stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4°C.**
5. Add required volume of ethanol (96- 100 %) to W1 Buffer and Wash Buffer when first open. **Store the solution at room temperature.**
6. Prepare a heating block or a water baths to 37 °C for step 4 and to 55°C for step 9 before operation.

General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Transfer $1 \sim 5 \times 10^6$ of cultures (fungal/ yeast cells) to a 1.5 ml microcentrifuge tube. (not provided)
2. Add 1 ml of **FA Buffer** to the cells and resuspend the cells by pipetting.
3. Descend the cells by centrifuging at $5,000 \times g$ for 2 min and discard the supernatant completely.
4. Resuspend the cells in **550 μ l of FB buffer and add 50 μ l of lyticase solution, mix well by vortexing**. Incubate the sample at 37°C for 30 min.
Caution: Lyticase solution and FB Buffer containing 14 mM of β -mercaptoethanol is hazardous to human health. perform the procedures involving Lyticase solution and FB Buffer in a chemical fume hood.
5. **(Optional)** If RNA-free genomic DNA is required, add 8 μ l of 50 mg/ml **RNase A** (not provided) and incubate for 2 min at room temperature.
6. Descend the cells by centrifuging at $5,000 \times g$ for 10 min. Remove the supernatant completely.
7. Add 450 μ l **TG1 Buffer** and mix well by pipetting. Transfer the sample mixture to a **bead tube**. (provided)
8. **Mix well by Plus-vortexing for 5 minutes.**
-- Elongate the pulse-vortexing time to 15~30 minutes if the sample cells are hard to be broken.
9. Add 20 μ l of **Proteinase K** (10 mg/ml) **and mix well by vortexing**. Incubate at 55°C for 15 min, vortex 30 seconds for every 5 minutes incubation.
10. Centrifuge the sample mixture at $5,000 \times g$ for 1 min and transfer 200 μ l of the supernatant to a new 1.5 ml microcentrifuge tube. (not provided)
11. Add 200 μ l of **TG2 Buffer** and mix well by pipetting.
12. Add 200 μ l of **ethanol** (96-100%) and mix well by pulse-vortexing for 10 seconds.
13. Place a **TG Mini Column** in **Collection Tube**. Transfer the sample mixture (including any precipitate) carefully to **TG Mini Column**. Centrifuge at $11,000 \times g$ for 30 second **then place the TG Mini Column to a new Collection Tube**.
14. Add 400 μ l of **W1 Buffer** to the **TG Mini Column**. Centrifuge at $11,000 \times g$ for 30 sec. Discard the flow-through and then place the TG Mini Column back to the Collection Tube.
--Make sure ethanol has been added into W1 Buffer when first use.
15. Add 750 μ l of **Wash Buffer** to the **TG Mini Column**. Centrifuge at $11,000 \times g$ for 30 sec. Discard the flow-through and then place the TG Mini Column back to the Collection Tube.
--Make sure ethanol has been added into Wash Buffer when first use.
16. Centrifuge at full speed ($\sim 18,000 \times g$) for an additional 3 min to dry the column.
Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
17. Place the **TG Mini Column** to **Elution Tube**.
18. Add 50 \sim 100 μ l of **Elution Buffer or ddH₂O** to the membrane center of the **TG Mini Column**. Stand **TG Mini Column** for 3 min.
Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
19. Centrifuge at full speed ($\sim 18,000 \times g$) for 1 min to elute total DNA.
20. Store total DNA at 4°C or -20°C .