Manual

FavorPrep™ Fungi/ Yeast Genomic DNA Extraction Mini Kit

Cat. No.: FAFYG 000
FAFYG 001
FAFYG 001-1
(For Research Use Only)

Kit Contents:

Cat. No:	FAFYG 000 (4 preps_sample)	FAFYG 001 (50 preps)	FAFYG 001-1 (100 preps)
Beads Tube	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	22 ml	45 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 x 5	550 µl x 10
Proteinase K [c]	1 mg	11 mg	11 mg x 2
TG Mini Column	4 pcs	10 pcs x 5	10 pcs x10
Collection Tube	8 pcs	100 pcs	100 pcs x 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_2O and **Store the solution at 4 °C.**			
ddH_2O volume for Proteinase K [c]	0.1 ml	1.1 ml	1.1 ml x 2

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1~ 5 $x10^6$
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 x g
2. 1.5 ml microcentrifuge tube
3. 96~100 % ethanol
4. Vortex
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 ß-mercaptoethanol is hazardous to human health. perform the procedures involving Lyticase solution and FB Buffer in a chemical fume hood.**
4. Add sterilized ddH_2O 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~ 5 x10^6 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 x g for 2 min and discard the supernatant completely.

4. Resuspend the cells in **550 µl of FB buffer and add 50 µl of lyticase solution, mix well by vortexing**. Incubat the sample at 37 °C for 30 min.
   Caution: Lyticase solution and FB Buffer containing 14 mM of ß-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 µl 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 x g for 10 min. Remove the supernatant completely.

7. Add 350 µl **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.**

9. Add 20 µl 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. Descend the cells by centrifuging at 5,000 x g for 1 min and transfer 200 µl of supernatant to a new 1.5 ml microcentrifuge tube. (not provided)

11. Add 200 µl of **TG2 Buffer** and mix well by pipetting.

12. Add 200 µl 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 x g for 30 second **then place the TG Mini Column to a new Collection Tube.**

14. Add 400 µl of **W1 Buffe**r to the **TG Mini Column.** Centrifuge at 11,000 x g for 30 seconds and discard the flow-through. 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 µl of **Wash Buffe**r to the **TG Mini Column.** Centrifuge at 11,000 x g for 30 seconds and discard the flow-through. 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 (~ 18,000 x 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 a **Elution Tube**.

18. Add 50 ~100 µl of **Elution Buffer or ddH$_2$O** 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 (~ 18,000 x g) for 1 min to elute total DNA.

20. Store total DNA at 4°C or -20°C.