User Manual

FavorPrep™ Fungi/Yeast Genomic DNA Extraction Mini Kit

(For Research Use Only)

Kit Contents:

Cat. No:	FAFYG 000 (4 preps)	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	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 (Liquid)	100 µl	1050 µl	1050 µl × 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
User Manual	1	1	1

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

Specification:

Principle:	Mini spin column (silica matrix)
Sample size:	1~5×10^6 cells
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. Block heater 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 required volume of ethanol (96~100%) to W1 Buffer and Wash Buffer for the first open. **Store the buffer at room temperature.**
5. Prepare block heater or water bath to 37°C for the step 4; 55°C for the step 9 before operation.

General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Transfer 1~5×10^6 of cultured cells (fungi/yeast) 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 × g for 2 minutes 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**. Incubate the sample at 37°C for 30 minutes.
   -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 minutes at room temperature.

6. Descend the cells by centrifuging at 5,000 × g for 10 minutes. Remove the supernatant completely.

7. Add 450 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.**
   -Elongate the pulse-vortexing time to 15~30 minutes if the sample cells are hard to be broken.

9. Add 20 µl of **Proteinase K and mix well by vortexing.** Incubate at 55°C for 15 minutes; vortex 30 seconds for every 5 minutes incubation.

10. Centrifuge the sample mixture at 5,000 × g for 1 minute and transfer 200 µl of the 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 × g for 30 seconds **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 × g for 30 secconds. 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 at the first use.

15. Add 750 µl of **Wash Buffer** to the **TG Mini Column.** Centrifuge at 11,000 × g for 30 secconds. 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 at the first use.

16. Centrifuge at full speed (~18,000 × g) for an additional 3 minutes 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 50~100 µl of **Elution Buffer or ddH$_2$O** to the membrane center of the **TG Mini Column**. Stand **TG Mini Column** for 3 minutes.
    -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 × g) for 1 minute to elute total DNA.

20. Store total DNA at 4°C or -20°C.