FavorPrep™ Fungi Genomic DNA Extraction HE Mini Kit

■ Kit Contents

Cat. No.	FAFG1030 (4 Preps)	FAFG1033 (50 Preps)	FAFG1034 (100 Preps)
FA Buffer	5 ml	60 ml	120 ml
FB Buffer	1 ml	8 ml	15 ml
FATG1 Buffer	1.5 ml x 2	40 ml	70 ml
FATG2 Buffer	4 ml	50 ml	100 ml
Wash Buffer (Concentrate)▲	1.5 ml	17.5 ml	35 ml
Elution Buffer	0.5 ml	5 ml	7 ml
Lyticase Solution	100 µl	550 µl x 2	550 µl x 4
RNase A Solution	130 µl	1250 µl	1250 µl x 2
Proteinase K (Liquid)	150 µl x 2	1050 µl x 3	1050 µl x 5
Bead Tubes	4 pcs	50 pcs	100 pcs
HE Column G	4 pcs	50 pcs	50 pcs x 2
HE Collection Tubes	4 pcs x 3	50 pcs x 3	100 pcs x 3
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of **Wash Buffer** by adding 96~100% ethanol.			
Volume of Ethanol for Wash Buffer▲	6 ml	70 ml	140 ml

1. All kit components are shipped at room temperature and should be stored at room temperature between 15~25˚C upon receipt, **except Lyticase Solution and RNase A Solution**.
2. Store Lyticase Solution and RNase A Solution at -20˚C upon receipt.

■ Specification

Format/Principle	Spin Column (silica matrix)
Binding Capacity	≤125 µg DNA/Column
Operation Time	<70 mins
Sample Size	≤250 mg
DNA Yield	≤30 µg
Elution Volume	30 µl

Procedure Overview

Fungi sample

- Transfer 100 mg of fungal sample into a 1.5 ml microcentrifuge tube.
- Add 100 µl of **FB Buffer** and 20 µl of **Lyticase Solution**.
- Incubate at 37°C for 20 mins.
- Add 24 µl **RNase A Solution** and incubate at 37°C for 10 mins.

Bead Tube

Centrifuge
12,000 xg, 2 mins

- Add 650 µl of **FATG1-PK mixture** and mix thoroughly.
- Transfer sample into a **Bead Tube**.
- Homogenize or vortex for 1 min.
- Incubate at 55°C for 10 mins.
- Centrifuge for 2 mins.

HE Column G

Centrifuge
12,000 xg, 1 min

- Transfer the supernatant (up to 700 µl) into an **HE Collection Tube**.
- Add 800 µl of **FATG2 Buffer** and 500 µl of ethanol, mix well.
- Transfer 950 µl of the mixture into an **HE Column G** and centrifuge.
- Repeat for the remaining mixture.

Centrifuge
12,000 xg, 1 min

- Add 900 µl of **Wash Buffer** (ethanol contained) and centrifuge.

Centrifuge
16,000 xg, 2 mins

- Add 500 µl of **Wash Buffer** (ethanol contained) and dry the column membrane.

Centrifuge
12,000 xg, 1 min

- Add 30 µl of **Elution Buffer**.
- Stand the column for 5 mins.
- Obtain purified gDNA.

■ Preparation Before Starting

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Additional materials: 96~100% ethanol.
3. (Optional) For long-term DNA storage, immerse the fungal sample in FavorPrep™ NApreserve Reagent (Cat. No. FNPR1084) as instructed in the user manual.
4. Set up a water bath or dry bath at 37°C and 55°C. Preheat the Elution Buffer to 55°C for elution step.
5. Set a vortexer to maximum speed, or the homogenizer to 2,500 rpm at 4°C.
6. Check **FATG1 Buffer** before use. If precipitates are observed, vortex FATG1 Buffer until precipitates are completely dissolved.
7. Fresh preparation of **FATG1-PK mixture**, premix 600 µl of **FATG1 Buffer** and 50 µl of **Proteinase K (Liquid)** before executing DNA extraction.
8. Add indicated volume of ethanol (96~100%) into **Wash Buffer**, mix well and store at room temperature.

■ Sample preparation

For Spore/Hypha/Yeast Samples

1. Transfer 100 mg (up to 250 mg) of fungal sample into a 1.5 ml microcentrifuge tube (not provided).
2. Proceed with the **General Protocol**.

For Fruiting Body Sample (Basidiocarp, Apothecium)

1. Cut the fruiting body sample into pieces smaller than 1 cm.
2. Transfer fungal sample into a 1.5 ml microcentrifuge tube (not provided) and add 1 ml of **FA Buffer** to rinse the surface of the sample.
3. Centrifuge at **5,000 xg** for 5 mins to remove the supernatant.
4. Grind 100 mg (up to 200 mg) of the fungal sample in liquid nitrogen to a fine powder and transfer the powder to a new microcentrifuge tube (not provided).
   - Do not allow the sample to thaw and continue immediately to step 5.
5. Proceed with the **General Protocol**.

General Protocol

- **Note:** All centrifugation steps should be performed at **12,000 ×g** at room temperature (except wash step).

1. Resuspend the sample in 100 µl of **FB Buffer** and add 20 µl of **Lyticase Solution**, mix well by vortexing.
2. Incubate the sample at 37°C for 20 mins.
3. Add 24 µl of **RNase A Solution** and incubate at 37°C for 10 mins.
4. Add 650 µl of **FATG1-PK mixture** and mix well by pipetting.
5. Transfer the samples to a **Bead Tube**, then homogenize or vortex for 1 min (for high molecular weight DNA) or 3 mins (for higher yield).
6. Incubate the mixture at 55°C for 10 mins and vortex occasionally during incubation.
7. Centrifuge for 2 mins, then transfer the supernatant (up to 700 µl) carefully into a new **HE Collection Tube**.
8. Add 800 µl of **FATG2 Buffer** and 500 µl of ethanol (96~100%), then mix thoroughly by pipetting.
9. Place an **HE Column G** into a new HE Collection Tube.
10. Transfer 950 µl of mixture carefully into the HE Column G and centrifuge for 1 min. Discard flow-through.
11. Repeat step 10 for the remaining mixture and place the HE Column G in a new HE Collection Tube.
12. Add 900 µl of **Wash Buffer** (ethanol contained) to the HE Column G. Centrifuge at 12, 000 xg for 1 min then discard flow-through.
13. Add 500 µl of Wash Buffer (ethanol contained) to the HE Column G. Centrifuge at 16,000 xg for 2 mins to dry the membrane directly. Discard flow-through and the HE Collection Tube.
14. Place the HE Column in an **Elution Tube**, then add 30 µl of prewarmed **Elution Buffer** or ddH_2O (pH 7.5~9.0) directly onto the membrane. Stand the HE Column for 5 mins.
    - **Important step!** For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.
15. Centrifuge for 1 min to elute DNA.