【Product Name】 MagPure Fungal HW DNA Kit

【Product specifications】 48 Preps, 96 Preps, 480 Preps

【Intended Use】

This product provides an automated solution for the preparation of high molecular weight DNA for fungal samples. It uses zirconia bead grinding to lyse fungal cell walls, which is suitable for extracting high-purity total DNA from liquid cultured fungi, mycelium, spore powder, trace fungi or bacteria, parasitic fungi and other samples. The obtained DNA can be directly used for down stream applications such as PCR, chip analysis, virus DNA detection, NGS, Nanopore sequencing etc.

【Main Composition】

Cat.No.	D638401	D638402	D638403
Purification Times	48 Preps	96 Preps	480 Preps
Bead Tubes F	48 tubes	96 tubes	5 x 96 tubs
RNase A	15 mg	30 mg	150 mg
RNase Dissolve Buffer	3 ml	3 ml	10 ml
MagPure Particles G2	1.5 ml	3 ml	15 ml
Buffer STL	40 ml	80 ml	350 ml
Buffer CXP	25 ml	50 ml	220 ml
Buffer GW1*	44 ml	88 ml	2 x 220 ml
Buffer GW2*	20 ml	50 ml	2 x 100 ml
Buffer GW3	50 ml	90 ml	450 ml
Elution Buffer	10 ml	30 ml	100 ml

【Storage conditions and Validity】

RNase A and MagPure Particles G2 should be stored at 2–8°C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

【Prefilled Plate/Single Strip Component】

Components		D6384-TL-06	D6384-S-48
Bead Tubes F		96 tubes	48 tubes
RNase A		30 mg	15 mg
RNase Dissolve Buffer		3 ml	3 ml
Buffer STL		70 ml	40 ml
TL-Tip		12	24
V bottom plate/ Reagent strip	Row 1/7: 400µl Buffer CXP	6 plates	48 strips
	Row 2/8: 750µl Buffer GW1		
	Row 3/9: 750µl Buffer GW1		
	Row 4/10: 750µl Buffer GW2 20µl MPG2		
	Row 5/11: 750µl Buffer GW3		
	Row 6/12: 100µl Elution Buffer		

【Preparation before Use】

- Add 0.6ml (48Preps), 1.2ml (96 Preps) or 6.0ml (480 Preps), Rnase Dissolve Buffer to the Rnase A to get a concentration at 25mg/ml, and store at -20~8℃ after dissolve.
- Dilute Buffer GW1with 56ml (48 Preps), 112ml (96 Preps) or 2 x 280ml (480 Preps) 100% ethanol and store at room temperature
- Dilute Buffer GW2 with 80ml (48 Preps), 200ml (96 Preps) or 2 x 400ml (480 Preps) 100% ethanol and store at room temperature

【 Protocol Part 1: Sample Preparation】

1. Sample preparation

- **Solid culture fungi:** Add 1.0~1.8ml normal saline or PBS to wash out the fungi from the solid culture medium, transfer to a 2.0ml centrifuge tube, centrifuge at 13,000 x g for 3 minutes to collect fungi, and discard the supernatant.
- **Liquid culture fungi:** Transfer 1.8ml culture medium (fungal wet weight should not exceed 100mg) to a 2.0ml centrifuge tube, centrifuge at 13,000 x g for 3 minutes to collect fungi, and discard the supernatant.
- **Spore powder:** Transfer 30~50mg spore powder to a 2.0ml centrifuge tube.
- **Parasitic microorganisms in body fluids:** Transfer 1.0~1.8ml serum, plasma, secretions, swab soaking solution, tissue homogenate, body fluids, etc. to a 2.0ml centrifuge tube, centrifuge at 13,000 x g for 10 minutes to collect fungi, and discard the supernatant.
- **Sputum:** Take an appropriate amount of sputum, add 4 times the volume of freshly prepared 0.1% DTT solution, vortex to mix for 10 seconds, and place at room temperature for 15~30 minutes, during which vortex and shake continuously until the mass disappears and the sputum is completely homogenized. Transfer 1.8ml solution to a 2.0ml centrifuge tube, centrifuge at 13,000 x g for 10 minutes to collect microorganisms, and discard the supernatant.
- **Whole blood:** Take 0.5ml whole blood into a centrifuge tube, add 3 times the volume of red blood cell lysis buffer (10 x Buffer RBC) to lyse red blood cells, centrifuge at 500 x g for 5 minutes to precipitate and remove white blood cells. Transfer 1.5~1.8ml supernatant to a 2.0ml centrifuge tube, centrifuge at 13,000 x g for 10 minutes to collect microorganisms, and discard the supernatant.
- **Parasitic fungi or bacteria in tissues:** Take 30~100mg frozen or fresh animal and plant tissues, add 1.0~1.5ml normal saline or PBS and homogenate thoroughly, place for 1~3 minutes to precipitate and remove large particles. Transfer the supernatant to a 2ml centrifuge tube, centrifuge at 13,000 x g for 10 minutes to collect microorganisms, and discard the supernatant.

2. **Add 600μl Buffer STL and 10μl RNase Solution to the sample, resuspend the precipitate by vortexing, then transfer all the resuspended solution to the Bead Tubes F,** screw tightly, vortex at maximum speed for 10 minutes on a vortex mixer, or perform rapid bead grinding on a bead grinder.
    - PowerLyzer grinder: recommend 2000rpm for 30s, pause for 30s and then repeat once.
    - FastPrep 24 grinder: recommend 5m/s for 30s, pause for 30s, and then repeat once.
    - Tissue Lysis II grinder: recommend 25Hz for 5mins, reposition and then repeat once.
3. Centrifuge at 13,000 x g for 3 minutes, follow the steps in Part 2/3.

【 Part 2: Manual Purify by Single tube】

1. **Transfer 500μl solution (from Part 1) to a 1.5 ml centrifuge tube.**
2. **Add 350μl Buffer CXP and 25μl MagPure Particles G2,** mix by inverting slowly for 15~30 times, place at room temperature for 10 minutes, during which invert and mix for several times. Place at magnetic stand for 1 minute, remove the supernatant.

   Before use, MagPure Particles G2 needs to be fully resuspended by shaking (1000~1200rpm) for 2 minutes.
3. **Add 750μl Buffer GW1, mix by pipetting for 10 times or incubate with shaking (800~1200rpm) for 1 minute.** Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
4. **Add 750μl Buffer GW1, mix by pipetting for 10 times or incubate with shaking (800~1200rpm) for 1 minute.** Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
5. **Add 750μl Buffer GW2, mix by pipetting for 10 times or incubate with shaking (800~1200rpm) for 1 minute.** Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
6. **Add 750μl Buffer GW2, mix by pipetting for 10 times or incubate with shaking (800~1200rpm) for 1 minute.** Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
7. **Do not remove the centrifuge tube from the magnetic stand, add 750μl Buffer GW3 slowly, do not disperse the magnetic beads, place for 60 seconds, and be careful to aspirate the supernatant.**
8. **Add 50~100μl Elution Buffer,** mix by pipetting for 10 times or incubate with shaking (800~1000rpm) for 5 minutes. Place the tube to the magnetic stand for 2 minutes. Transfer the supernatant containing the purified DNA to a new centrifuge tube.

【 Part 3: Auto Purify by 16/32 channel nucleic acid extractor 】

1. Bottled reagents: add the reagents to the 96 well plate following the above table of prefilled kit contents.
   Prefilled reagents: invert the 96 well plate to suspend the magnetic beads completely. Pat the plate to make reagents fall back to the bottom of plate. Stay the plate at table for 1 minute, remove the sealing pack and sealing film.
2. Add 400μl of mixture (from Part 1) to each well of row 1/7.
3. Insert the magnetic tip and 96-well plate in to the machine (hole A1 is placed at the left inner corner). Turn on the machine and start the program.
4. About 30 minutes, extraction finish.
5. Take out the 96 well plate and magnetic tip comb.
6. Transfer DNA into a 1.5ml centrifuge tube and store at -20~8℃.

【 Program recommendation for Magen MagMix 16/32 extractor 】

No.	Name	Well	Volume	Mix		Wait		Magnet			Magnet	Heat	
				Time	Speed	Time	Position	Up/Down	Surface	Bottom		Plate	Temp
1	Magnet	4	500	30s	8	0	0	60s	0	0	Auto	/	/
2	Bind	1	750	300s	7	0	0	90s	0	0	Auto	/	/
3	Wash1	2	600	90s	8	0	0	60s	0	0	Auto	/	/
4	Wash2	3	600	90s	8	0	0	60s	0	0	Auto	/	/
5	Wash3	4	600	60s	8	0	0	60s	0	0	Auto	/	/
6	Wash4	5	600	0	8	0	0	60s	0	0	Auto	/	/
7	Elute1	6	100	180s	8	0	0	0	0	0	Auto	6	55
8	Elute2	6	100	420s	6	0	0	90s	0	40	Auto	6	55
9	Remove	3	500	30s	8	0	0	0	0	0	Auto	/	/