【Product Name】 MagPure Bacterial 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 bacterial 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.	D638301	D638302	D638303
Purification Times	48 Preps	96 Preps	480 Preps
Lysozyme	90 mg	180 mg	800 mg
Protease Dissolve Buffer	5 ml	10 ml	50 ml
RNase A	15 mg	30 mg	150 mg
Proteinase K	30 mg	60 mg	300 mg
MagPure Particles G2	1.5 ml	3.0 ml	15 ml
Buffer P1	30 ml	60 ml	350 ml
Buffer SDS	1.8 ml	5 ml	20 ml
Buffer CXP*	28 ml	56 ml	210 ml
Buffer GW1*	44 ml	88 ml	2 x 220 ml
Buffer GW2*	20 ml	50 ml	2 x 100 ml
Buffer BW3	50 ml	90 ml	400 ml
Elution Buffer	10 ml	30 ml	120 ml

【Storage conditions and Validity】

Lysozyme, Proteinase K, 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		D6383-TL-06	D6383-S-48
RNase A		30 mg	15 mgl
Proteinase K		60 mg	30 mg
Buffer SDS		5 ml	1.8 ml
Buffer P1		60 ml	30 ml
Lysozyme		180 mg	90 mg
Protease Dissolve Buffer		10 ml	5 ml
TL-Tip		12	24
V bottom plate/ Reagent strip	Row 1/7: 400µl Buffer CXP	6 plates	48 strips
	Row 2/8: 600µl Buffer GW1		
	Row 3/9: 600µl Buffer GW1		
	Row 4/10: 600µl Buffer GW2 25µl MPG2		
	Row 5/11: 600µl Buffer BW3		
	Row 6/12: 120µl Elution Buffer		

【Preparation before Use】

- Add 1.8ml (48Preps), 3.6ml (96 Preps) or 16ml (480 Preps), Protease Dissolve Buffer to the Lysazyme to get a concentration at 50mg/ml, and store at -20~8℃ after dissolve
- Add 0.6ml (48Preps), 1.2ml (96 Preps) or 6.0ml (480 Preps), Protease Dissolve Buffer to the Rnase A to get a concentration at 25mg/ml, and store at -20~8℃ after dissolve.
- Add1.5ml (48Preps), 3.0ml (96 Preps) or 15ml (480 Preps), Protease Dissolve Buffer to the Proteinase K to get a concentration at 20mg/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
- Add 12ml (48 Preps), 24ml (96 Preps) or 90ml (480 Preps) 100% isopropanol to Buffer CXP, mix well by inverting and store at room temperature.

【 Protocol Part 1: Sample Preparation】

1. Sample preparation

- **Fermentation broth or culture broth (negative bacteria)**: Transfer 1.0~2.0ml bacterial culture broth or fermentation broth (<2 x 10^9 bacteria) into a 2.0ml centrifuge tube, centrifuge at 5,000 x g for 10 minutes to collect bacteria, and discard the culture broth. Add 500μl Buffer P1, vortex to resuspend the bacteria, add 25μl Buffer SDS and 25μl Proteinase K, vortex to mix well, and incubate at 65°C for 20 minutes.
- **Fermentation broth or culture broth (positive bacteria):** Transfer 1.0~2.0ml bacterial culture broth or fermentation broth (<2 x 10^9 bacteria) into a 2.0ml centrifuge tube, centrifuge at 5,000 x g for 10 minutes to collect bacteria, and discard the culture broth. Add 500μl Buffer P1 and 30μl Lysozyme, incubate with shaking (900rpm) at 37°C for 30~120 minutes. Add 25μl Buffer SDS and 25μl Proteinase K, incubate at 65°C for 20 minutes.
- **Tissue samples:** Take 50~200mg tissue samples, homogenize thoroughly with physiological saline or PBS, centrifuge at 500 x g for 10 minutes to remove somatic cells. Transfer the supernatant to a new centrifuge tube, centrifuge at 5,000 x g for 10 minutes to collect bacteria, and discard the supernatant. Add 500μl Buffer P1 and 30μl Lysozyme, incubate with shaking (900rpm) at 37°C for 30~120 minutes. Add 25μl Buffer SDS and 25μl Proteinase K, incubate at 65°C for 20 minutes.
- **Secretions, soaking solutions, body fluids, etc.:** Take 1.0~2.0ml secretions, sputum, serum, plasma, blood, swab soaking solutions or other body fluid samples into a 2.0ml centrifuge tube, centrifuge at 13,000 x g for 3 minutes to collect bacteria, and discard the supernatant. Add 500μl Buffer P1 and 30μl Lysozyme, and incubate with shaking (900rpm) at 37°C for 30~120 minutes. Add 25μl Buffer SDS and 25μl Proteinase K, incubate at 65°C for 20 minutes.
- **Dry swab sample:** Transfer the swab to a 2ml centrifuge tube, add 500μl Buffer P1 and 30μl Lysozyme, and incubate with shaking (900rpm) at 37°C for 30~120 minutes. Add 25μl Buffer SDS and 25μl Proteinase K, incubate at 65°C for 20 minutes.
- **Difficult to lyse bacteria (bead grinder):** Transfer 1.0~2.0ml bacterial culture or fermentation broth (<2 x 10^9 bacteria) into a 2.0ml centrifuge tube, centrifuge at 5,000 x g for 10 minutes to collect bacteria, and discard the culture broth. Add 550μl Buffer P1 and 300~500mg acidic glass powder (0.1-0.2mm, separately ordered), vortex at maximum speed for 5~10 minutes or grind on a bead grinder for 90~150 seconds. Place for 2 minutes, transfer 500μl supernatant to a new centrifuge tube, add 25μl Buffer SDS and 25μl Proteinase K, incubate at 65°C for 20 minutes.

2. Add 10μl RNase Solution, mix well, and place at room temperature for 10~20 minutes.
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 400μl Buffer CXP, mix by inverting for 10 times, incubate at 50°C for 3~5 minutes until the precipitation disappear.
3. Add 25μl MagPure Particles G2, mix by inverting for 10~15 times, place at room temperature for 3 minutes, during which invert and mix for several times. Place at magnetic stand for 1~2 minutes, remove the supernatant. Vortex for an instant and aspirate the supernatant again.
4. Add 750μl Buffer GW1 and vortex for 10 seconds. Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
5. Add 750μl Buffer GW1 and vortex for 10 seconds. Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
6. Add 750μl Buffer GW2 and vortex for 10 seconds. Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
7. Add 750μl Buffer GW2 and vortex for 10 seconds. Place the tube to the magnetic stand for 1 minute. Then remove the supernatant.
8. Do not remove the centrifuge tube from the magnetic stand, add 750μl Buffer BW3 slowly, do not disperse the magnetic beads, place for 60 seconds, and be careful to aspirate the supernatant.
9. Add 80~100μl Elution Buffer, gently tap to drop the magnetic beads from wall and resuspend in Elution Buffer. Incubate with shaking (600~800rpm) at 55°C for 10 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 500μl 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	Mix	1	900	120s	6	0	0	0	0	0	Auto	1	50
2	Wash	2	750	10s	8	0	0	0	0	0	Auto	1	50
3	Magnet	4	750	20s	8	0	0	90s	0	0	Auto	1	50
4	Bind	1	900	250s	7	0	0	120s	0	0	Auto	/	/
5	Wash1	2	750	60s	8	0	0	90s	0	0	Auto	/	/
6	Wash2	3	750	60s	8	0	0	90s	0	0	Auto	/	/
7	Wash3	4	750	60s	8	0	0	60s	0	0	Auto	/	/
8	Wash4	5	750	0	8	0	0	60s	0	0	Auto	/	/
9	Elute1	6	100	180s	7	0	0	0	0	0	Auto	6	55
10	Elute2	6	100	300s	6	0	0	90s	0	40	Auto	6	55
11	Remove	3	500	30s	8	0	0	0	0	0	Auto	/	/