BACTERIA DNA EXTRACTION KIT

Kit Components

Components	Storage
Bacteria Enzyme Buffer	RT
Bacteria Lysis Buffer	RT
Proteinase K	2 - 8ºC
Proteinase K Buffer	2 - 8ºC
RNase A	2 - 8ºC
Bacteria MagNa Mix	RT
Bacteria Wash Buffer 1	RT
Bacteria Wash Buffer 2	RT
Bacteria Elution Buffer	2 - 8ºC
MagNa Stand (optional)	RT

Materials needed prior to start

- Add 96 - 100% Ethanol to Wash Buffers as indicated on the bottle.
- Set the water bath/heat block at 56°C and 37ºC.
- Reconstitute Proteinase K with Proteinase K Buffer and store at 2 - 8ºC.
- Reagents not supplied with kit: 1X PBS (pH 7.4), Lysozyme stock (20mg/ml, to be prepared freshly)

PROTOCOL

Sample Preparation

1. Take 1 ml of overnight grown bacterial culture (OD_{600} > 1) and centrifuge at 10,000 rpm for 5 minutes at room temperature. Discard the supernatant.
2. Add 500 µl of **1X PBS** and resuspend the pellet completely by pipette mixing.
3. Centrifuge at 10,000 rpm for 5 minutes at RT and discard the supernatant.

For Gram Positive pellet proceed to step 4.

For Gram Negative pellet proceed to step 14.

Gram Positive Bacterial Lysate Preparation

4. To the pellet, add **190 µl of Bacteria Enzyme Buffer** and completely resuspend the pellet by pipette mixing (*ensure proper resuspension of pellet*).
5. Add **10 µl of freshly prepared lysozyme** and mix thoroughly by pipette (*pipette mixing 3 - 4 times*).
6. Incubate at **37ºC for 60 minutes.**
7. Add **800 µl of Bacteria Lysis Buffer** and mix thoroughly by pipette (*pipette mixing 10 - 15 times*).
8. Add **20 µl of Proteinase K** and quick vortex.
9. Incubate at **56ºC for 60 minutes** (*pipette mix completely every 30 minutes*).

Note: For better dispersion, use 200 µl pipette for re-suspending the pellet

10. Add **10 µl of RNase A** and quick vortex.
11. Incubate at room temperature for 15 minutes.
12. Centrifuge at **14,000 rpm for 5 minutes** at room temperature.
13. Transfer the supernatant to a fresh 1.5 ml tube. Proceed to step 21 (DNA binding and washing)

Gram Negative Bacterial Lysate Preparation

14. Add **1 ml of Bacteria Lysis buffer** to the pellet and resuspend the pellet by pipette mixing (*pipette mix 10 - 15 times*).
15. Add **20 µl of Proteinase K** and mix well by quick vortex.
16. Incubate at **56ºC for 60 minutes** (*pipette mix 10 - 15 times for every 30 minutes*).
17. Add **10 µl of RNase A** and vortex the tube for 30 seconds.
18. Incubate at room temperature for 15 minutes.
19. Centrifuge at **14,000 rpm for 5 minutes** at room temperature**.**
20. Transfer the supernatant to a fresh 1.5 ml tube. Proceed to step 21 (DNA binding and washing)

DNA Binding and Washing

Note: Vortex the Bacteria MagNa Mix to ensure complete dispersion of the particles.

21. Add **400 µl** of **Bacteria MagNa Mix** to the lysate. Gently mix the contents by inverting the tube for 8 - 10 times.

22. Incubate at room temperature for 5 minutes.
23. Place the tube on MagNa Stand for 5 minutes at room temperature.
24. Carefully discard the supernatant without removing the tube from the MagNa Stand (*Make sure the pellet is not disturbed*).
25. Add **500 µl** of **Bacteria Wash Buffer 1** and remove the tube from MagNa Stand.
26. Resuspend the pellet by pipette mixing for about 10 - 12 times (*Ensure complete dispersion of the particles*).

Note: For better dispersion, use 200 µl pipette for re-suspending the pellet

27. Place the tube back on MagNa Stand. Keep it until the solution becomes clear (*30 – 60 seconds*).
28. Carefully discard the supernatant without removing the tube from the MagNa Stand (*make sure the pellet is not disturbed*).
29. Add **500 µl** of **Bacteria Wash Buffer 2** and gently invert mix the tube without removing from MagNa Stand for 5 - 6 times (*Surface wash only*).
30. Discard the supernatant without removing the tube from MagNa Stand.
31. Repeat the steps 29 - 30.
32. Air dry the pellet with the tube on MagNa Stand at room temperature for 10 - 15mins.

Note: Do not over dry the pellet.

DNA Elution

33. After drying, remove the tube from MagNa Stand.
34. Add **50 - 100 µl of Bacteria Elution Buffer** and resuspend the pellet by pipette mixing (*pipette mix 10 - 15 times*).
36. Incubate at **56°C for 10 minutes** with intermittent tapping.
37. Place the tube on MagNa Stand for 5 minutes or until the solution appears clear.
38. Carefully transfer the supernatant containing DNA to a sterile 1.5 ml micro centrifuge tube without removing the tube from MagNa Stand (*make sure the pellet is not disturbed*).
39. Discard the MagNa particles.

Note: In the elution step, if the MagNa particles take more than 10 minutes for clearing, then spin the tubes at 14,000 rpm for 5 minutes, place on MagNa Stand until solution clears and then collect the supernatant for pure DNA.

Downstream applications

DNA obtained from this method is ready to use for any downstream application without any further precipitation or purification step.

Troubleshooting guide

Observation	Possible causes	Suggested Solution
Low DNA Yield or Poor Quality	Low Culture Density	Use bacterial culture with OD_{600} above 1.
		Avoid using old cell cultures. For better yield, use overnight cell culture.
	Poor Cell Lysis	Cells may not be dispersed properly in lysis buffer. Disperse the cells by adequate pipette mixing. Ensure complete dispersion of lysate by pipette mixing every 30 minutes during lysis incubation.
		For bacterial strains with high DNA content, the lysate may appear viscous. For such samples thorough pipette mixing while lysis incubation and before 'binding & washing' step is recommended.
		Add required volume of Proteinase K into the Lysis buffer. Proteinase K solution should be stored at 2 - 8°C.
	Ethanol is not added to wash buffers	Add 100% ethanol to wash buffers as suggested on the bottles.
RNA contamination	RNase A not added	Add RNase A as per the protocol.