

Additional Protocol

Phase Lock Gel (PLG)

Isolation of genomic DNA from *Staphylococcus aureus*

Note: Please be sure to read Phase Lock Gel Manual before starting.

Equipment and reagents to be supplied by user

- Buffer A: 50 mM Tris-HCl, 50 mM EDTA (pH 8.0)
- Lysozyme/RNase mixture: 500 µl lysozyme (100 mg/ml), 25 µl RNase (10 mg/ml)
- Proteinase K (10 mg/ml)
- Sodium dodecyl sulfate (SDS), 10%
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- Isopropanol
- TE buffer: 10 mM Tris-HCl, 1 mM EDTA (pH 8.0)
- 7.5 M ammonium acetate
- 99% ethanol
- Phase Lock Gel Heavy (200 x 1.5 ml tubes) (Ref. No. 2302810)

Procedure

1. Prepare a standard overnight culture of *Staphylococcus aureus*
2. Harvest cells from 6 ml sample of the overnight culture and resuspend in 500 µl of buffer A.
3. Add 20 µl of a lysozyme/RNase mixture and incubate for 30 min at 37°C.
4. Add 50 µl of proteinase K and 20 µl of 10% SDS and incubate at 56°C until the lysate clears.
5. Extract the lysate twice with phenol:chloroform:isoamyl alcohol in pre-spun 1.5 ml Phase Lock Gel Heavy tubes to separate the phases.
6. Precipitate DNA by adding 400 µl of isopropanol and collect by centrifugation at 5,000 x g for 5 min.
7. Redissolve DNA in 300 µl of TE buffer.
8. Reprecipitate for 30 min at 4°C by adding 100 µl of 7.5 M ammonium acetate and 800 µl of 99% ethanol and collect by centrifugation at 5,000 x g for 5 min.
9. Dry DNA and dissolve again in TE buffer.

Ordering Information

Ref. No.	Product Name	Quantity	Sample Volume	Tube Color
2302810	Phase Lock Gel Heavy 1.5 ml – 200 Tubes	200	100 – 500 µl	green
2302830	Phase Lock Gel Heavy 2 ml – 200 Tubes	200	100 – 750 µl	yellow
2302850	Phase Lock Gel Heavy 15 ml – 100 Tubes	100	1 – 6 ml	† clear
2302870	Phase Lock Gel Heavy 50 ml – 25 Tubes	25	5 – 20 ml	† clear

† PLG Heavy is opaque while PLG Light is translucent.

Phase Lock Gel tubes are also available in different sizes and formulations for handling of different sample volumes and using different organic extraction solvents.

[Please find all our Phase Lock Gel products on our website.](#)