

Post-alkaline Extraction for Western Time-course Experiments

Solutions

Sample buffer

- 0.06 M Tris-HCl pH 7.0
- 5% glycerol
- 2% SDS
- 4% β -mercaptoethanol
- Few grains of bromophenol blue

Can be kept at room temperature but after a month check that β -mercaptoethanol is okay by smelling buffer.

Procedure

1. Collect 2.5 OD and spin down.
2. Resuspend in 200 μ l dH₂O.
3. Incubate 3 min at room temperature.
4. Spin 15 s at 12000 rpm and discard supernatant.
5. Resuspend in 70 μ l sample buffer.
6. Boil 3 min.
7. Spin 15 s at 12000 rpm and retain supernatant in new tube (can be frozen and reboiled) or loaded immediately (cool for a bit, never chill).
8. Load about 5 μ l per lane.