

SOP: SP029

Preparation of electrocompetent *M. smegmatis*

Materials and Reagents:

1. *M. smegmatis*, 1 ml frozen stock or growing culture
2. Biosafety cabinet (note 1)
3. 51 ml sterile LB media
4. 10% sterile glycerol
5. 20% sterile tween-80
6. Sterile aerosol resistant pipette tips, 200 μ l
7. Pipetman, 200 μ l
8. Disposable inoculating loops
9. 37°C shaking incubator
10. Spectrophotometer, visible light
11. Spectrophotometer cuvettes
12. Sterile 1.7 ml eppendorf tubes
13. Cryostorage box
14. Freezer, -80°C
15. Biohazard bags
16. Autoclave tape
17. Autoclave
18. 50 ml conical Falcon tubes
19. Disposable plastic serological pipettes, 50 ml and 5 ml
20. Pipette aid
21. Allegra 6 R centrifuge
22. Sterile 250 ml Erlenmeyer flask
23. Ice bucket and ice

Protocol:

1. _____ Grow a fresh 50 ml culture of *M. smegmatis* MC²155 in either Middlebrook 7H9-ADS-Tween or LB-Tween (0.05%) to mid-log phase (OD⁶⁰⁰ = 0.5 to 1.0).
2. _____ Incubate cells on ice for at least 10 minutes but no longer than two hours. (note 2)
3. _____ Transfer cells to chilled 50 ml conical tube.
4. _____ Spin down cells in Allegra 6 R at 3000 rpm at 4°C for 10 minutes. Decant supernatant from pellet into autoclavable container.
5. _____ Resuspend pellet in 40 ml cold sterile 10% glycerol.
6. _____ Centrifuge at 3000 rpm at 4°C for 10 minutes in Allegra 6R tabletop centrifuge. Decant supernatant into autoclavable container.
7. _____ Resuspend pellet in 40 ml cold sterile 10% glycerol.
8. _____ Centrifuge at 3000rpm at 4°C for 10 minutes. Decant supernatant into autoclavable container.
9. _____ Resuspend pellet in 5 ml ice cold 10% glycerol and store on ice.
10. _____ Distribute cells in 400 μ l aliquots into 1.7 ml eppendorf tubes and store at -70°C for up to one year.
13. _____ Autoclave and dispose of all liquid waste generated.

Notes:

1. All work should be performed in a Biosafety cabinet in order to prevent contamination. See SOP SP041 for use of the biosafety hood.
2. For the following steps, keep cells as close to 0°C (on ice) as possible.