Preparation of Competent Cells of *M. bovis* BCG V.1

Jonathan Rey A Ibale[1]

[1]School of Life Sciences, CUHK

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Jonathan Rey Ibale

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This protocol is a standardized protocol obtained from the Polytechnic University of Hong Kong and is in current use in SC 193 Laboratory under Prof. Au Wing Ngor for M. bovis BCG experimentation. The cells produced thereof are electrocompetent.

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1 3h

Prepare a fresh 7H9 broth for every culture step. The 7H9 broth is prepared as follows:

1. Mix together:
   - 0.47 g 7H9 broth powder
   - 0.1 g casitone (pancreatic digest of casein)
   - 0.40 g sodium pyruvate
   - 200 µL glycerol
   - 90 mL nuclease-free double-distille water (MQ water)
2. Autoclave mixture at **121 °C** for 10 mins, and shake media gently after.
3. Cool to about **45 °C** , then add **10 mL** OADC and 1 mL 10% sterile Tween-80. (Sterile 10% Tween-80 is prepared by diluting 1 mL Tween-80 in 9mL sterile MQ water, then sterilized by filtration in 0.22µm membrane.)
4. The 7H9 broth may be stored in **4 °C** after preparation and can be used for up to two weeks.

2 1w 1d

Inoculate a starter bacterial stock to 100 mL 7H9 broth (0.1g casitone, 0.44 g sodium pyruvate) incubate in **37 °C** for 7-8 days without shaking. (Prolonged incubation causes dehydration so seal the flask/tube during incubation.)

3 1w 1d

Aliqout 1 mL from the primary culture into a fresh, pre-warmed 100 mL 7H9 broth and incubate in **37 °C** for 8 days, followed by another 8 days of incubation with shaking at 90 rpm. Measure absorbance in 600nm (OD600) before shaking and periodically during dynamic incubation phase.

1d

When the absorbance OD600 reaches 0.4-0.5, add 0.1 volume of autoclaved 2M glycine, and

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4 incubate for another 16-24 hours. Perform a contamination check along with this incubation using BHI media pre-warmed and incubated in **37 °C** . If the inoculation develops turbidity overnight, the culture is contaminated with non-specific bacteria or rapidly growing mycobacterium (RGM).

5 3h

Aliquot bacterial suspension in 4 50-mL conical tubes (around 25 mL each) and centrifuge at 3000*xg* for 10 mins in RT. Remove media and wash cells, i.e. resuspend, centrifuge, then remove media, three (3) times with autoclaved and pre-warmed 10% glycerol first at 20 mL, 10 mL, then 5 mL. Resuspend the final pellet in 500 µL 10% glycerol (2% aliquot volume) and aliquot 200µL into sterile propylene tubes for storage in **-80 °C** .

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