



Phagehunting Program

Media and Reagents for Growing *Mycobacterim smegmatis*

Mycobacterium smegmatis mc² 155, smeg, is the host bacterium. It is a common soil organism, non-pathogenic. It grows slowly (colonies in ~ 4 days). It is resistant to carbenicillin (CB), so we always include CB at 50 µg/ml to kill other bacteria. Cyclohexamide (CHX) at 10 µg/ml is added to the media because it kills most molds and yeast. In liquid culture, *M. smegmatis* tends to clump. We grow it from a colony from a plate in 7H9 media with calcium, CB/CHX/ADC, and 0.05% Tween® 80, then subculture this to media without the Tween® for cells for phage infection. We grow it in standard test tubes placed at an angle on a shaker, but for bigger volumes, we use baffled flasks. Most of the phages have a calcium requirement. We always put 1mM CaCl₂ in media and top agar. We standardly use 0.35% top agar in our screens, hopefully to identify bigger phages that might not form plaques on the standard 0.7% agar.

ADC (NO HEAT, filter sterilize)

60 g dextrose
25.5 g NaCl
150 g Albumin
2850 ml ddH₂O

20% Tween® (50°C to dissolve, filter sterilize)

20 ml Tween® 80
80 ml ddH₂O

MBTA (Middlebrook Top Agar) made at 0.7% melted, diluted to 0.35% with 7H9 (plus 2 ml CaCl₂*)

4.7 g 7H9
7 g agar
H₂O to 900 ml

Phage Buffer (autoclave or filter sterilize, add 0.1mM CaCl₂* prior to use)

10ml 1M Tris, pH 7.5
10 ml 1M MgSO₄
4 g NaCl
980 ml ddH₂O

7H9 (autoclave, add antibiotics, ADC, and calcium prior to use)

4.7 g 7H9 broth base
5 ml 40% glycerol
900 ml ddH₂O

7H10 Plates (Autoclave, cool to 55° C and add 100 ml ADC and 10 ml 0.1 mM CaCl₂ , CB, CHX and then pour.)

19g 7H10 agar

12.5 ml 40% glycerol

890 ml ddH₂O

CaCl₂ * Calcium is added to Mycobacterial growth media to ensure adequate calcium is available for necessary cellular metabolic processes. Note that for the most part, we add calcium to obtain a 0.1mM concentration in the final solutions of each media or reagent we use. (Therefore we will add 1ml of the 1mM CaCl₂ stock solution to phage buffer, but 2ml of CaCl₂ to 7H9(because it will be used to dilute top agar by 50%.)

7H9 + CB + CH + ADC + CaCl₂ - for liquid culture of smeg

100ml 7H9

10 ml ADC

100 µl of CB

100 µl of CHX

250 µl Tween® (For intial sub-culture ONLY!) See direction below.

Growing *Mycobacterium smegmatis* mc²155

When culturing *Mycobacterium smegmatis* mc²155: Start by retrieving a sample from the frozen stock in the -70°C freezer and streaking it on a 7H10 ADC CB CHX plate. Allow to grow for several days. The goal is to produce isolated colonies. Smeg has a distinct colony morphology. When sub-culturing from this plate, pick a tiny (smaller than what you can see) piece from the center of the colony to grow in liquid culture. The liquid media (7H9 ADC CB CHX) has Tween® added to minimize the clumping of the bacterial growth. Once a homogenous culture is obtained, subculture in media without Tween®.

For growing smeg: Initial transfer from plated smeg

(Autoclave, for each L, add 100 ml ADC enrichment and CaCl₂ to 1mM, 100 µl carbenicillin (CB) and 100 µl cycloheximide (CHX). Store in fridge after adding ADC.) When growing smeg from plate, add 250 µl 20% Tween® 80 to 100 ml bottle. It is advisable to use a ‘touch’ of the culture from your plate into 1-2 ml of this Tween®-media. Vortex well. Place on shaker for at least 24 hours. Sub-culture from this culture into media without Tween® for phage infection.

For growing smeg for infections (7H9 without Tween®)

Transfer a small amount (1 – 100 µl of the smeg grown in Tween® into a flask that is only filled ~1/5 full of 7H9 + CaCl₂ + ADC + CB + CHX (same concentrations as above but no Tween®). Place securely on shaker for 24 hours.