

Consumables/Reagents (See online media preparation guides)

- Agar Plates
- 7H9 (90 mL/100 mL culture)
- ADC (10 mL/100 mL culture)
- 0.1M CaCl₂ solution (1 mL/100 mL culture)
- CB solution (100 µL/100 mL culture)
- CHX solution (100 µL/100 mL culture)
- Tween®80 solution (250 µL/100 mL culture)
- Appropriate micropipette tips
- Inoculating loop or sterile equivalent
- Serological pipettes

HELPFUL TIPS

- *If culture agitation ceases before it has reached stationary phase, biofilms may form. Biofilm formation will lead to clumping *smeg* in bacterial lawns.
- Mycobacteria are named for their mycolic acids—long chain fatty acids branching from their cell walls that give them a waxy coating. If given the chance, the cells will stick together and form small clumps that appear as pellicles. Overtime, large clumps form and the culture will not be usable for plating. Tween®80 (a detergent and nutrient source) is used to minimize the clumping.
- Streak for isolation from the first culture received. This is done to maintain a stable, retrievable culture in the lab. Use single isolated colonies for amplifying *smeg*.
- Starter liquid cultures propagate the dry, wrinkled colonies of *smeg* into a smooth, milky, non-clumping liquid culture. Addition of Tween®80 is the most successful way to do this.
- *smeg* liquid cultures are the working cultures used for isolation, purification, and amplification of mycobacteriophage.
- Excellent aseptic technique is necessary for continued success.
- Learning the smell, color, and surface appearance of *smeg* is essential for success in growing *smeg*. Cultures of *smeg* are very stable and can be maintained for an extended period of time.

PROCEDURES

1. In order to grow *M. smegmatis* mc²155 on a plate, streak for isolation using a small inoculum from the liquid culture received. You will always want to start your culture from a single clone. Use best practices of aseptic technique.
 - a. Streak for isolation using standard protocol, one of which is the [T-streak](#) method.
 - b. Incubate for at least three days at 37°C until several isolated colonies have grown before picking. They should be light tan colored with a waxy consistency. Alternatively, this step can be performed at room temperature, though it will take more time for colonies to grow. You may need to parafilm the plates as you incubate to deter loss of moisture.

- c. Parafilm plates and store at 4°C for continued use.
2. Prepare initial liquid cultures from plate stocks (7H9 WITH Tween®80).
 - a. As mentioned above, *smeg* tends to clump when grown in liquid culture. To combat this, initial stocks grown from plates should be grown in growth media plus Tween®80. This stock is considered to be the “First Generation” of growth, and should be used to make working stocks of *smeg*.

- b. The recipe is as follows:

To 1 bottle 7H9 (90 mL), add:

10 mL ADC

1 mL 0.1M CaCl₂

100 µL CB

100 µL CHX

250 µL Tween®80

This mixture should be stored at 4°C.

- c. If access to a shaking incubator is available, aliquot 2 – 5 mL of the above medium into a sterile test tube and inoculate with a touch of a colony. Incubate this shaking at 37°C overnight.
 - d. If access to a shaking incubator is not available, this step can also be performed by aliquotting 5 mL of the media into a 25 mL non-baffled sterile Erlenmeyer flask with a stir bar, then inoculating with a touch of a colony, and stirring at room temperature over a longer period of time (>2 days). This culture can be saved at 4°C for use in multiple subcultures.
3. Prepare *smeg* culture for phage infections (follow above recipe without Tween®80).
 - a. When growing *smeg* for general plating, omit Tween® to optimize phage infection. To prepare this growth media, simply follow the above recipe, but do not add Tween®80. Any amount between 5 – 500 mL can be grown at a time.
 - b. Obtain a baffled Erlenmeyer flask that is **5 times the volume** of the culture to be prepared. For example, use a 25 mL flask for a 5 mL culture.
 - c. This culture is inoculated with 10 – 100 µL of culture from the original Tween®80 stock. The culture can be incubated shaking at 37°C overnight, or using a stir bar and stir plate, as described in step #2c and #2d.