

## TITERING PHAGE

Kay Finn

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Soft Agar: 4 g Nutrient broth  
2.5 g NaCl  
3.25 g Agar  
add 1 stir bar, 500 ml H<sub>2</sub>O, in Schott bottle, sterilize

Dilution Fluid:0.5 g Tryptone  
3.5 g NaCl  
0.25 g MgSO<sub>4</sub>  
500 ml H<sub>2</sub>O in 1 liter Schott bottle  
sterilize 40 minutes; after cooling, attach to sterile dispensette.

Carefully dilute phage with pipetters, be sure to use fresh tips every time and **do not** touch the tubes with pipetters. Mix dilutions well with out spilling contents. Use sterile 13x100mm tubes with stainless steel caps. Be sure to heat soft agar to boil and stir before taking 2.5 ml out per dilution plate sample + 1 for the control. Temper the soft agar tubes to ~50 degrees for at least 5 minutes before using to plate. Use Dispensette to make accurate dilutions of phage:

Dilute the Phage stock down in the following manner:  
50ul stock into 5 ml of dilution fluid, mix ..... 10E2  
50ul of above into 5ml of dilution fluid, mix ..... 10E4  
50ul of above into 5 ml of dilution fluid, mix ..... 10E6  
50ul of above into 5 ml of dilution fluid, mix ..... 10E8  
50ul of above into 5 ml of dilution fluid, mix .....10E10

Add 2 drops of plating bacteria and then 0.2 ml of the 10E10 dilution to one of the tempered tubes of soft agar, mix gently and pour quickly onto LB agar plate, swirl to get even coverage before the agar sets up. *10.2 Plate*

Add 2 drops of plating bacteria and then 0.5 ml of the 10E10 dilution to one of the tempered tubes of soft agar, repeat as above *10.5 Plate*

Add 2 drops of plating bacteria and then 0.7 ml of the 10E10 dilution to one of the tempered tubes of soft agar, repeat as above *10.7 Plate*

Control is only 2 drops of plating bacteria to one of the tempered tubes of soft agar, and continue as above.

**Do not disturb** until set....about 20 minutes, then put in incubator 30 or 37 degrees for overnight.

In morning count plaques.