K Agar Plates

		For 1 L Fleaker (750ml)	
2.36g	KCl	**1.77g**	**KCl**
3.0g	NaCl	**2.25g**	**NaCl**
2.5g	Bacto-peptone	**1.88g**	**Bacto-peptone**
17.0g	Bacto-agar	**12.75g**	**Bacto-Agar**

(Makes 1L, = approx. 40 plates)

1. Combine into a 2L flask with 1000ml de-ionized/distilled water and a stir bar. Cover with double - thick foil, autoclave, and then stir the flask on a stir plate. When it=s cool to the touch, add the following:

    a. 1.0ml cholesterol solution (0.1g cholesterol / 10ml undenatured ethanol, heat to dissolve) or **0.75ml for 750ml**
    b. 1.0ml 1M $CaCl_2$ for 1L **or 0.75ml for 750ml**
    c. 1.0ml 1M $MgSO_4$ for 1L **or 0.75ml for 750ml**

2. Mix well on a stirring plate and aliquot about 10 ml into medium size (6 cm) plates. It is easier to have a water bath set up at 55ºC and place the flask there between every 10 to 12 aliquots (without using water bath it would be difficult to pour all the plates before the agar solidifies).
3. Allow agar to solidify in plates at room temperature, stack them within their original plastic packaging bags and store upside-down in refrigerator until ready for use. One liter agar should yield ~70 medium sized plates.

* It is best to prepare and pour the agar all on the same day, but if time is limited, the agar (**with cholesterol, etc. not yet added**) can be kept in a 50ºC water bath overnight. This will keep the agar liquefied, and the remaining ingredients can be added the next day after cooling.

OP50 Broth (an *E. coli* stock solution) To prepare 50ml of broth

0.25g NaCl
0.5g Bacto-peptone
50ml dH2O

Aliquot into test tubes (about 5 ml each). Autoclave. After cooling inoculate (using sterile technique) a loop of *E. coli* culture into the broth.

Incubate for 24hrs at 37°C.

K + OP50 plates

1. OP50 must be spread onto the previously described K agar plates once they have hardened. This is done by transferring 90 ul drops of OP50 solution onto 6 cm agar plates with a sterile pipette.
2. Next, the drops are spread evenly onto the plate with a sterile spreader. If using glass spreader, it is sterilized by first dipping it into distilled de-ionized water, then in ethanol, and then burning the ethanol off in a flame. Alternatively, disposable L-spreaders work well.
3. Once spread, the plates are labeled and incubated at about 37^{o}C for at least 24 hrs to establish a bacterial lawn.
4. After lawn is established parafilm and transfer the plates to a fridge (store upside-down).
5. Always use plate with sufficient OP50 count.