

Kanamycin stock is kept in the 4°. The stock is 10 mg/mL. Final concentration of kan for both plates and broth is 30 µg / mL.

Remember, kanamycin is sensitive to heat! Don't autoclave it or add it to anything hot.

Anything that goes into the autoclave should have a LOOSE CAP -- this is to ensure that pressure can escape and your stuff does not explode. DO NOT TIGHTLY CAP ANYTHING THAT GOES IN THE AUTOCLAVE.

Making LB broth

> Use distilled water from the specified spigot in the sink. Measure out desired amount of water in glass container.

> Use the LB powder in the big container on top of the microwave. Measure out an appropriate amount of grams per mL. (It's 25 grams per litre.)

> Add LB powder to water and microwave briefly to break up large clumps.

> Place a piece of autoclave tape on the lid of your glassware. Bring up to the autoclave and use the liquid setting for 20 minutes.

> When the autoclave cycle is complete, bring the broth down to cool. Set aside any LB to which you don't want to add kanamycin. When the broth has cooled down sufficiently, add appropriate amount of kanamycin from the fridge.

Making kan plates

> Use distilled water from the specified spigot in the sink. I use about 20 mL of broth per plate, so a sleeve of 25 plates comes out to 500 mL of water.

> Add the LB Agar, which is NEXT TO, **NOT** ON TOP OF the microwave. Calculate the appropriate amount of LB Agar to add (it's 35 grams per litre, so for 500 mL that's 17.5 grams of agar.)

> Microwave briefly to break up big clumps.

> Put a stir bar in your broth. (Don't add stir bar before the microwave step -- the stir bar has metal in it.)

> Put a lid loosely on the glassware and put a piece of autoclave tape on the glassware.

> Bring up to the autoclave and use the liquid setting for 20 minutes.

> When autoclave cycle is complete, bring the broth down to cool. When the broth has cooled down sufficiently, add appropriate amount of kanamycin from the fridge. BE CAREFUL HERE -- there is a sweet spot at which the broth has cooled sufficiently to add kanamycin but has not solidified yet. This is when the stir bar comes in handy -- you can keep the liquid moving by placing it on a magnetic plate as it cools down.

> Use an electric pipette (or pour) your plates out.