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Preparation of 100mg/ml kanamycin solution

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Kanamycin is an aminoglycoside antibiotic which binds the 30S ribosomal subunit; causes mis-translation. It is used for the selection of cells containing Kanamycin-resistant plasmids on plates and on liquid media at a working concentration of 50-100 µg/mL.

It is most often prepared as a 1000x working stock of 50 -100 mg/mL that is stored at -20 °C.

The kanamycin solution is measured and then mixed into a container of liquid or plate media before it has solidified. Cells are then streaked onto the plates or grown in the liquid media. Only cells containing the plasmids resistant to kanamycin will grow.

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Kanamycin, Stocks, Cell Culture

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Materials

- Disinfection solutions (freshly prepared 10% bleach and 70% alcohol)
- Kanamycin sulphate powder (CAS#: 70560-51-9; Linear Formula: $C_{18}H_{36-37}N_{4-5}O_{10-11} \cdot H_2SO_4$)
- Distilled or deionised water

Equipment

- Electronic balance
- Freezer

Consumables

- Weighing boat
- Spatula
- 10ml Measuring cylinder
- 0.22µm syringe filter
- Sterile 20 ml syringe
- Sterile 50 ml centrifuge tube
- Sterile 1.5 ml microcentrifuge tubes
- p1000 Micropipette
- Sterile 1000 µl Pipette tips
- Waste containers

Kanamycin sulphate is a hazardous substance ([SDS](#)) and may damage fertility or the unborn child. It is highly recommended to weigh out all antibiotics in a fume hood or powder weighing station.

Preparing 1000x kanamycin sulphate stock solution

20m

- 1 Clean work surfaces and work space using freshly prepared **10 % (v/v)** bleach, followed by **70 % (v/v)** alcohol.
- 2
 - Using the electronic balance, carefully weigh out **0.4 g** of kanamycin sulphate powder (CAS#: 70560-51-9; Linear Formula: $C_{18}H_{36-37}N_{4-5}O_{10-11} \cdot H_2SO_4$) into an appropriate size weighing boat using a spatula.



Kanamycin sulphate is a hazardous substance ([SDS](#)) and may damage fertility or the unborn child. It is highly recommended to weigh out all antibiotics in a fume hood or powder weighing station.

- 3 Carefully tip the powder into a sterile **50 mL** centrifuge tube.

- 4 Using a **10 mL** measuring cylinder, measure out **4 mL** of sterile distilled or deionised water.
- 5 Pour **2 mL** water into the **50 mL** centrifuge tube containing the kanamycin powder.
- 6 Use the remaining **2 mL** to rinse the weighing boat and pouring that into the **15 mL** falcon tube to make **4 mL** total and mix gently by inversion.
- 7 Using the sterile **20 mL** syringe, aspirate the solution from the falcon tube
- 8 Affix a sterile 0.22 μm micro filter to the end of the syringe and dispense the solution into a new sterile **15 mL** centrifuge tube.
- 9 Pipette **1000 μL** of the filtered **100 mg/mL** Kanamycin solution into 4 x sterile **1.5 mL** microcentrifuge tubes.
- 10 Close the tubes, label and store them at **-20 °C** if they are not to be used immediately. This is a 1000x stock of **100 $\mu\text{g}/\mu\text{L}$** Kanamycin which is the normal working concentration in liquid or solid media. The stock can be diluted before use to reach the desired concentration.

Kanamycin sulphate does degrade with time and there can be differences in potency and stability between batches. It is recommended when starting with a new batch to titrate the concentration to ensure 100% of non-resistant bacteria are killed. If the antibiotic properties start to drop off, ideally make a new batch from scratch or titrate to an increased effective concentration.