

**Protocol #XXX : Preparation of M9 minimum medium (0.4% glucose)
iGEM Grenoble-EMSE-LSU**

Simon Pacouret (simon.pacouret@phelma.grenoble-inp.fr)

July 2013 (Source: Stéphane Pinhal (advisor))

Material and reagents:

- Deionized H₂O
- M9 Minimal Salts, 5X (SIGMA, M6030-1kg)
- CaCl₂ (1M)
- MgSO₄ (1M)
- B1 Vitamin (1%)
- Glucose (20%)
- 1000X trace elements solution:
 - 200μL H₂O
 - 100μL Na₂-EDTA-2H₂O
 - 100μL ZnSO₄-7H₂O
 - 100μL CoCl₂-6H₂O
 - 100μL MnCl₂-4H₂O
 - 100μL H₃BO₃
 - 100μL Na₂-MoO₄-2H₂O
 - 100μL CuSO₄-5H₂O
- FeSO₄ (anhydrous)
- 0.22-0.45 μm filter system (syringe or stericup)

Protocol:

- 1) Dissolve 56.4g M9 minimum salts 5X in 1L deionized water and autoclave for 15min at 121°C. This solution can be stored at RT for a few weeks.
- 2) Prepare the following mix:
 - 340mL deionized water
 - 100mL autoclaved M9 minimum salts 5X
 - 50μL CaCl₂ 1M
 - 1mL MgSO₄ 1M
 - 250μL B1 Vitamin 1%
 - 10mL Glucose 20%
- 3) Filter the mix. This 500 mL filtered M9 solution can be stored in the dark, at 4°C, for a few days
- 4) The day of your experiment, dissolve 0.03g FeSO₄ in 1mL sterile deionized water²
- 5) Supplement 50 mL filtered M9 solution with 5 μL FeSO₄ and 45 μL 1000x trace elements solution.