# Bee9: An M9 derived medium for bee gut bacteria

## Description

This protocol is used to prepare stock solutions for an M9-based ‘minimal’ medium containing trace metals and vitamins, without casamino acids. Additional vitamins, and growth nutrients are added for growth of *S. alvi* and/or *Gilliamella* strains.The pH should be adjusted to 5.5

## 1. Preparation of HMB (hutner’s mineral base) modified metals 44 (component 1)

* Weight on a balance the following:

1. 1.095g ZnSO4 x 7H2O, dissolve in **5ml** ddH2O at pH=2

2. 0.914g FeSO4 x 7H2O, dissolve in **5ml** ddH2O at pH=2

3. 0.154g MnSO4 x H2O, dissolve in **5ml** ddH2O at pH=2

4. 0.392g CuSO4 x 5H2O, dissolve in **100ml** ddH2O at pH=2

5. 0.248g Co(NO3)2 x 6H2O, dissolve in **100ml** ddH2O at pH=2

6. 0.177g Na2B4O7 x 10H2O, dissolve in **100ml** ddH2O at pH=2

* Mix **1.**, **2.**, **3.**, and add 10ml of each of **4.**, **5.**, **6**. to obtain **component 1**.
* Adjust volume to 100ml and sterile-filter with 0.22 um filter
* Keep it at 4C, light protected

## 2. Preparation of additional salts (components 2, 3, 4, 5)

* **Component 2:** 1.72g of MgSO4 x 7H2O, dissolve in **100ml** ddH2O at pH=2, sterile-filter with 0.22 um filter
* **Component 3:** 3.33g of CaCl2 x 2H2O, dissolve in **100ml** ddH2O at pH=2, sterile-filter with 0.22 um filter
* **Component 4:** 0.99g of FeSO4 x 7H2O, dissolve in **100ml** ddH2O at pH=2, sterile-filter with 0.22 um filter
* **Component 5:** 0.974g of (NH4)6Mo7O24 x4H2O, dissolve in **100ml** ddH2O at pH=2, sterile-filter with 0.22 um filter
* Keep at RT, light protected

## 3. Preparation of the vitamin 10x stock solution (component 6a)

* 0.2g of Calcium pantothenate, dissolve in **10ml** ddH2O
* 0.01g of Thiamine-HCl, dissolve in **10ml** ddH2O
* 0.01g of biotin, dissolve in **10ml** ddH2O
* Mix 79ml of ddH2O, 10ml of **2.**,10ml of **3.**, and 1ml of **4.**
* Sterile-filter the final 100 ml of vitamin stock solution (component 6a)
* Component 6a can be kept for at least 6 months at 4**°**C in the fridge, protected from light

## 5. Preparation of 10x stock solution of M9 (component 8)

* Mix the following:
* 60g Na2HPO4
* 30g KH2PO4
* 5g NaCl
* 10g NH4Cl
* Add water to 1l, autoclave
* Keep at RT

## 6. Preparation of *S. alvi* specific vitamins

* Weigh out powder of the following compounds and dissolve in M9 base to a concentration of 10 mM (for final concentration of [0.1 mM]
* 4-Hydroxybenzoate
* P-aminobenzoic acid
* Pyridoxine\*HCl
* Choline

## 7. Prepare 500ml of M9 base medium

* Prepare the final M9 base medium freshly on the day of use
* For 500ml mix the following:
* Component 1: 500 μl
* Component 2: 8390 μl
* Component 3: 1000 μl
* Component 4: 100 μl
* Component 5: 10 μl (100 uL of 1/10 component 5 for small M9 volume)
* Component 6a (10x Vitamin stock): 50ml
* Component 8 (10x M9 stock): 50ml
* Custom vitamins (10mM stock) 5 mL
* ddH2O: 385 ml

## 8. Prepare carbon + media

* Dissolve compounds of choice in M9 to a final concentration of 10 mM. Citrate is a good single carbon source. Glucose [10 mM] can also be added for *Gilliamella* growth.
* Common substrates include.
  + Alpha-ketoglutaric acid
  + Fumaric acid
  + Malic acid
  + Succinic acid
  + Citric acid
  + Sodium pyruvate
  + Sodium acetate
  + Sodium lactate
* Check pH and adjust to 5.5 to 6