

iGEM2015 – Microbiology – BMB – SDU	
Title: M9 minimal medium SOP number: SOP0001_v01 Version number: v01	Date issued: 2015.05.14 Review date: 2015.05.14 Written by: EMT, KBS

1. Purpose

To make M9 minimal medium.

1. Area of application

Selection for knock outs

1. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
2 containers (5 mL)	Micro Storage	• •	
1 glass bottle (250 mL)	Micro Storage	• •	
1 glass bottle (1 L)	Micro Storage	• •	
2 glass bottles (100 mL)	Micro Storage	• •	
Autoclave	Laboratory 1. Floor	• •	
Sterile filter	Micro Storage	• •	
		• •	

1. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Na ₂ HPO ₄ x 7H ₂ O		Contact lab manager	Basement hallway	
KH ₂ PO ₄		Contact lab manager	Basement hallway	
NaCl		Contact lab manager	Basement hallway	
NH ₄ Cl		Contact lab manager	Basement hallway	
MgSO ₄		Contact lab manager	Basement hallway	
CaCl		Contact lab manager	Basement hallway	
Glukose or Glycerol		Contact lab manager	Basement hallway	
LA media		Contact lab manager	Basement hallway	

1. QC – Quality Control

1. List of other SOPs relevant to this SOP

1. Environmental conditions required

1. Procedure

1. Mix 5xM9 salts in 1 liter sterile water:

- | | | |
|---|---|---------|
| 1. Na ₂ HPO ₄ x 7H ₂ O | → | 64 g. |
| or Na ₂ HPO ₄ x 2H ₂ O | → | 42.5 g. |
| 2. KH ₂ PO ₄ | → | 15 g. |
| 3. NaCl | → | 2.5 g. |
| 4. NH ₄ Cl | → | 5 g. |

2. Carbon source
 1. If glukose (20%) → 20 mL per liter
 2. If glycerol (50%) → 20 mL per liter
3. Mix LA see SOP5
4. Autoclave products from 1 and 3
5. Filter 2 through 0.22 µm sterile filter
6. Solution of 1M MgSO₄ 2 mL per liter
7. Solution of 1M CaCl 0.1 mL per liter
8. After autoclavation mix 1, 2 and 3 to a final volume of 1 liter
 1. 750 mL sterile water
 2. 200 mL 5xM9
 3. 2 mL MgSO₄
 4. 20 mL carbon source
 5. 0.1 mL CaCl
 6. Fill up with sterile deionized water to a final volume of 980 mL
9. Make plates

1. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
One used Plastic		GMO	Yellow GMO Trash

1. Time consumption

- Total-time 3,5 hours.
- Hands-on-time 1 hour.

1. Scheme of development

Date / Initials	Version No.	Description of changes
2015.05.14/EMT + KBS	01	The SOP has been written

1. Appendices