Preparation of media

PROTOCOLS IGEM TU EINDHOVEN

EINDHOVEN UNIVERSITY OF TECHNOLOGY

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Biomedische Technologie

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Content

1 M9-based minimal media

Estimated bench time: 30 minutes
Estimated total time: 3 hours
Purpose: Preparation of media used for bacterial growth in phage experiments.

1.1 Materials

- Autoclaved 5x M9 salts
- 1 M $MgSo_4$
- 100 mM $CaCl_2$
- 1.5 M MgCl
- 3 g/L $FeSo_4$
- 100 g/L glucose
- Sterile deionized H_2O
- 0.22-µM filter

1.2 Setup & protocol

This medium is based on Santos, et al. (2014). PloS one, 9(7); and Sambrook J, Russell DW (2001) Molecular Cloning: A Laboratory Manual with some slight adaptations of our own.

- 1L 5x M9 salts should be prepared as followed:

Compound	Amount (g)
$Na_2HPO_4{\cdot}2H_2O$	37.6
KH_2PO_4	15
NaCl	2.5
NH_4Cl	5

- The 5x M9 salts must be sterilized by autoclaving.
- The medium (100 mL) should be formed as in the table below. The $MgSo_4$, MgCl, $CaCl_2$ and glucose should be prepared separately and sterilized by passing through a 0.22-µm filter before adding to the diluted M9 salts to prevent precipitation.

Compound	Amount
5x M9 salts	20 mL
1 M $MgSO_4$	200 µL
100 mM $CaCl_2$	100 µL
1.5 M MgCl	333 µL
3 g/L $FeSo_4$	100 µL
100 g/L glucose	5 mL
Sterile deionized H_2O	Up to 100 mL

- The medium should be made on the day of use and fresh medium should be made daily.