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# Preparation of GS2 medium

## Overview

Liquid GS2 medium consists of three components: the GS2 base medium, the GS2 salts, and the carbon source. The three components are prepared separately, sterilized, and then combined just before inoculation with cells. GS2 agar is made by adding 15 g/L agar to liquid GS2 before autoclaving.

## Prepare GS2 base medium

- Add the reagents shown in Table I to a 2 liter bottle

**Table. I.** Reagents to prepare 1 liter of GS2 base medium.

| **Reagent** | **Grams per liter (g/L)** | **Sigma product number** |
|---|---|---|
| KH2PO4 | 1.5 | P5655 |
| K2HPO4 | 2.9 | P3786 |
| Urea | 2.1 | U0631 |
| L-cysteine HCl | 2 | C6852 |
| MOPS | 10 | M3183 |
| Sodium citrate 2H2O | 3 | S4641 |
| Yeast extract | 6 | Y1625 |

- Add 900 mls milliQ water to the bottle.
- Add 1ml of an 0.1% resazurin (sigma R7017) solution to the bottle containing GS2 reagents. Resazurin is a redox sensitive dye that becomes colored in the presense of oxygen. Upon addition of resazurin, medium will turn blue/pink. Store resazurin solution at 4C.
- Adjust pH to 7 with potassium hydroxide. This requires adding about 6.5 ml 6N potassium hydroxide per liter of GS2 medium.
- Autoclave bottle of GS2 base medium and store on bench until needed

# Prepare 10x GS2 salts

- Add the reagents in Table II to a 2 liter bottle.

  **Table II.** Reagents to prepare 1 liter of 10X GS2 salts.

| Reagent | Grams per liter (g/L) | Sigma product number |
|---|---|---|
| MgCl2-6H2O | 10 | M2670 |
| CaCl2-2H2O | 1.5 | C5080 |
| FeSO4-7H2O | 0.0125 | F6833 |

- Autoclave GS2 salts and store on bench until needed

# Prepare 10% sugar solution

- In a 50 ml falcon tube, dilute 2.5 grams sugar with 25 mls milliQ water
- 0.2 micron filter sterilize
- Store filter sterilized solution at 4C

# Combine GS2 components

- To make 10 mls GS2 medium:
  - 9 ml GS2 base medium (autoclaved)
  - 1 ml 10X GS2 salts (autoclaved)
  - 0.3 ml 10% sugar solution (filter sterilized)