

Brock media for *S. acidocaldarius*

Corresponding author: Fredrik Hurtig

List of authors: Fredrik Hurtig, Gabriel Tarrason Risa and Buzz Baum

Group leader: Buzz Baum

Institution: MRC Laboratory for Molecular Cell Biology

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Abstract

This protocol describes how to prepare Brock growth media, and how to create glycerol stocks, for *Sulfolobus acidocaldarius.*

Related publication: Brock et al. 1972

Background

*S. acidocaldarius* belongs to the TACK superphylum (Guy and Ettema, 2011). At the time of writing, these archaea are the closest archaeal relatives of eukaryotes that can be easily grown and studied in a lab. As such, there is considerable interest in understanding their cell biology. *S. acidocaldarius* is also one of the most well established model systems for studying hyperthermophiles.

The growth medium is based on the first account of growing conditions for *Sulfolobus* by Brock and colleagues (Brock et al. 1972). It should be prepared fresh with dd water the same day you do the experiment, and adjusted to pH 2.9 with 1:1 sulfuric acid. However, Brock stock solutions can be stored after sterilisation at room temperature. The ferric solution should be protected from light to prevent chemical precipitation.

Materials

Stock solutions

* **Brock I (1L, 100x)**
	+ 7 g CaCl2 x 2H2O
	+ Autoclave
* **Brock II+III (1L, 100x)**
	+ 130 g (NH4)2SO4
	+ 25 g MgSO4 x 7H2O
	+ 28 g KH2PO4
	+ 50 ml trace element solution (from a **2000x TES**)
	+ 1.5 ml 1:1 H2SO4
	+ Autoclave
* **Fe-solution (1L, 100x)**
	+ 2 g FeCl3.6H2O
	+ Filter-sterilise
* **NZ-amine solution (1L, 10%, 100x)**
	+ 100 g NZ-Amine (Brand of NZ-Amine is important, we use Sigma-Aldrich 82524)
	+ Autoclave
* **Trace element solution (1L, 2000x)**
	+ 9.0 g Na2B4O7.10H2O (Insoluble at neutral pH, add 1:1 H2SO4 until it dissolves. May take several mL, however end pH is unimportant.
	+ 0.44 g ZnSO4.7H2O
	+ 0.1 g CuCl2.2H2O
	+ 0.06 g NaMoO4.2H2O
	+ 0.06 g VOSO4.2H2O
	+ 0.02 g CoSO4.7H2O (MW 281) (alternatively use CoCl2.6H2O, MW 237)
	+ 3.6 g MnCl2.4H2O
* **1:1 H2SO4 (100 mL)**
	+ 50 ml 95-98% H2SO4
	+ Slowly add H2SO4 to 50 ml dd H2O. The solution can become extremely hot and may need cooling!

Brock media preparation

|  |  |  |  |
| --- | --- | --- | --- |
|  | **For 1 L** | **For 250 mL** | **For 50 mL** |
| Brock I | 10 mL | 2.5 mL | 500 uL |
| Brock II + III | 10 mL | 2.5 mL | 500 uL |
| Fe solution | 10 mL | 2.5 mL | 500 uL |
| Sucrose 20% | 10 mL | 2.5 mL | 500 uL |
| NZ-amine (10%) | 10 mL | 2.5 mL | 500 uL |
| 1:1 H2SO4 | 200 uL | 50 uL | 10 uL |

Protocol

1. To start a new culture, take a small amount of frozen glycerol stock on a yellow pipette tip, and place the tip in ~20 ml room temperature Brock media, and grow at 75-80°C with rotation
	1. Culture normally reaches exponential phase in 24-48 hours
	2. Use aluminium foil to seal the opening of the flask tightly to prevent evaporation. A failure to do this can easily evaporate a culture overnight
2. Subsequent dilutions are needed every 24 hours
	1. Dilutions should be done using pre-heated Brock media

# **Glycerol stocks**

1. Grow cell to late exponential phase (OD 0.5-0.8, optional)
2. Centrifuge 10 ml of culture in a 15 ml falcon tube for 5 min at approximately 4000 RCF
3. Remove 9 ml of supernatant and resuspend the pellet in the remaining media. Add up to 1 ml of 100% glycerol and mix thoroughly.
4. Store at –80°C immediately.



Figure 1. Growth curves of *S. acidocaldarius* at various temperatures. *Reprinted with permission (Tarrason Risa, 2021).*

Additional notes

Brock media pH will increase significantly during growth if not enough acid has been added. In our experience pH 2.9 is usually sufficient for a stable pH during growth

Competing interests

The authors declare that they have no conflict of interest.

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References

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