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🌐 Fermentor Growth of *Streptococcus sanguinis*

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ABSTRACT

Streptococcus sanguinis is a lactic acid-forming bacterium that can be cultured in both aerobic and anaerobic conditions. It is a primary colonizer of the oral cavity but can also cause a heart disease called infective endocarditis. Our main objective for this protocol was to grow large, controlled cultures before and after manganese depletion for various analyses. In order to obtain large scale, reproducible growth of *S. sanguinis*, a biostat was used to maintain controlled conditions. After optimization, it was determined that traditional chemostat conditions (Burne and Chen, 1998) were not appropriate for these experiments. Thus, we decided on this modified protocol. Using this method, we were able to identify a concentration of the non-specific metal chelator, EDTA, that would lead to a decreased growth rate in a manganese transporter mutant but not in the wild-type strain.

ATTACHMENTS

[20180808_160423.jpg](#) [20180808_160257.jpg](#) [20180808_155550.jpg](#) [20180808_155418.jpg](#)

GUIDELINES

These instructions are specific to a Sartorius Stedim Biostat® B with a 1.5 L capacity UniVessel® Glass + BioPAT® Fundalux, DO probe, and pH probe. Adjustments may be required if using different equipment.

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

Created: Aug 25, 2020

PROTOCOL integer ID:
41016

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biostat, Streptococcus,
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depletion

MATERIALS

- ✂ RNeasy Mini Kit **Qiagen Catalog #74104**
- ✂ RNase-Free DNase Set **Qiagen Catalog #79254**
- ✂ Bacto™ Brain Heart Infusion **Thermo Fisher Catalog #237300**
- ✂ Antifoam 204 **Sigma Catalog #A8311**
- ✂ Potassium Hydroxide **Fisher Scientific Catalog #1310-58-3**
- ✂ Sodium Hydroxide **Fisher Scientific Catalog #1310-73-2**
- ✂ Hydrochloric Acid **Fisher Scientific Catalog #7647-01-0**
- ✂ EDTA 0.5 M pH 8.0 **Invitrogen Catalog #AM9261**
- ✂ RNa protect Bacteria Reagent **Qiagen Catalog #76506**
- ✂ DNA-free DNA Removal kit **Invitrogen Catalog #AM1906**
- ✂ Needleless Injection Site Swabbable Female Luer Lock to Barb Connector **Qosina Catalog #80210**

STEP MATERIALS

- ✂ RNa protect Bacteria Reagent **Qiagen Catalog #76506**
- ✂ RNeasy® Mini Kit **Qiagen Catalog #74104**
- ✂ RNase-Free DNase Set **Qiagen Catalog #79254**
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BEFORE START INSTRUCTIONS

1. The afternoon before the run, start a 40 mL pre-culture at 37 °C and appropriate oxygen concentration with antibiotics (if appropriate).
2. Make 5 L of BHI with 25 Parts per Million (PPM) antifoam. Autoclave 02:00:00 .
3. Set up fermentor according to manufacturer's specifications/user's needs.

When running an experiment for the first time, test to see how long it takes for the media to flow from the carboy to the vessel at the chosen flow rate (will vary depending on the length of the tubing used).

Inoculation

- 1 Set up fermentor to experiment specifications: pO₂ set to 5% with 0.03 lpm max air flow; nitrogen set at 0.08 lpm; stirrer set at 250 rpm; 37 °C 7.4 800 mL

Equipment	
Biostat® B	NAME
Benchtop Bioreactor	TYPE
Sartorius	BRAND
N/A	SKU

Equipment	
UniVessel® Glass 1.5 L capacity	NAME
Vessel	TYPE
Sartorius Stedim	BRAND
N/A	SKU
https://www.sartorius.com/en/products/fermentation-bioreactors/benchtop-bioreactors/univessel-glass	LINK

Equipment	
BioPAT® Fundalux	NAME
Optical density probe	TYPE
BioPAT	BRAND
N/A	SKU

Equipment	
EasyFerm Plus PHI VP 120 Pt100	NAME
pH probe	TYPE
Hamilton	BRAND
238633-1111	SKU

Equipment	
InPro6800/12/160/4112111	NAME
Dissolved oxygen probe	TYPE
Mettler Toledo	BRAND
5230580	SKU

2 Remove 15 mL media from vessel to incubate 37 °C as a check for contamination.

2.1 Remove 500 µL media and store at -80 °C for metabolomics analysis.

3 Take OD₆₀₀ reading using 1 mL of 40-mL overnight pre-culture.

Equipment

Genesys 150

NAME

UV-VIS Spectrophotometer

TYPE

Thomas Scientific

BRAND

1160V96

SKU

4 Centrifuge remaining volume 39 mL of overnight culture.

4303 x g, 4°C, 00:10:00

4.1 Decant supernatant and resuspend in 15 mL BHI.

4.2 Inoculate into vessel using 20-mL syringe.

Ramping up air flow

5 As the absorbance units (AU) increase, gradually ramp up the air flow.

5.1 When AU reaches 0.10, turn off the DO control and set air flow to 0.03 lpm.

5.2 When AU reached 0.30, set air flow to 0.20 lpm.

Note

If the experiment is meant to be low oxygen, skip this step.

- 5.3** When AU peaks and begins to drop, set air flow to 0.50 lpm. Turn on media pumps: input at 17% and output at 34%.

Note

For our fermentor, 17% ~ 700 mL h⁻¹

Note

If the experiment is meant to be low oxygen, do not increase the air flow.

Expected result

SK36 (wild type) growth should not be drastically affected by the addition of EDTA. For the primary Mn transporter mutant ($\Delta ssaACB$), the OD will increase initially and then should start to drop in OD (by 0.01 AU) ~38 min after EDTA addition.

Experimental Conditions

- 6** Before proceeding with sample collection, allow culture to adjust to media flow for 01:00:00 .
- 7** At 1 h post-media flow (T₋₂₀), remove sample 40 mL from vessel using a syringe (usually 60 mL capacity) using the sampling port.

- 8 At the predetermined time, add EDTA to [M] 100 micromolar (μM) to carboy in [A] 5 mL BHI using a syringe into the inoculation port.

[X] EDTA 0.5 M pH 8.0 Invitrogen Catalog #AM9261

Note

Using the flow time calculated previously (see *Before Starting*), determine how long it will take for media to flow from carboy to vessel and subtract from 20 min. For example, if it takes 4 min to flow from carboy to vessel, add EDTA 16 min after the first sample was removed.

- 9 At T_0 (20 min after first sample), add EDTA to [M] 100 micromolar (μM) to vessel in [A] 5 mL BHI using a syringe into the inoculation port.

[X] EDTA 0.5 M pH 8.0 Invitrogen Catalog #AM9261

- 10 Collect the remaining samples at T_{10} , T_{25} , and T_{50} .

Sample Collection Protocols

- 11 For metabolomics samples, aliquot [A] 30 mL of cell culture into conical tube.

- 11.1 Swirl immediately in a dry ice/ethanol bath for [T] 00:01:00 .

- 11.2 Centrifuge sample immediately.

[S] 4303 x g, 4°C, 00:05:00

11.3 Aliquot 500 µL of supernatant media and store at -80 °C .
Remaining volume can be decanted or stored at -20 °C for hydrogen peroxide quantification or other analysis.

11.4 Store cell pellet at -80 °C until ready for analysis.

12 For RNA samples, set up tubes for each sample containing 4 mL RNAProtect.
RNAProtect Bacteria Reagent **Qiagen Catalog #76506**

12.1 Add 2 mL of cell culture to RNAProtect tubes. Vortex for 00:00:05 .

12.2 Incubate at Room temperature for at least 00:05:00 but less than 03:00:00 .

12.3 Centrifuge cell culture in RNAProtect 4303 x g, 4°C, 00:10:00 .

12.4 Store at -80 °C until ready to isolate RNA.

12.5 Isolate RNA and remove DNA.

RNeasy® Mini Kit **Qiagen Catalog #74104**

⊗ RNase-Free DNase Set **Qiagen Catalog #79254**

⊗ DNA-free DNA Removal kit **Invitrogen Catalog #AM1906**

12.6 Store RNA at -80 °C until ready for analysis.