

May 04, 2023

Version 2

🌐 Artificial saliva V.2

📁 In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.3byl4k1jzvo5/v2>

Bjørn Peare Bartholdy¹, a.g.henry¹

¹Leiden University

BYOC



Bjørn Peare Bartholdy

Delft University of Technology, Leiden University



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Protocol Citation: Bjørn Peare Bartholdy, a.g.henry 2023. Artificial saliva. **protocols.io**

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Protocol status: Working

We use this protocol and it's working

Created: December 10, 2021

Last Modified: May 04, 2023

Protocol Integer ID: 55830

Keywords: artificial saliva solution for oral biofilm growth, oral biofilm growth, artificial saliva solution, artificial saliva, oral biofilm, dental calculus

Funders Acknowledgements:

ERC European Union's Horizon 2020

Grant ID: STG-677576

Abstract

Creating an artificial saliva solution for oral biofilm growth, specifically dental calculus.

This is a modified version of Sissons et al. 1991.

Guidelines

This protocol explains how to mix  1000 mL of artificial saliva.



Materials

Chemicals

Mucin from porcine stomach (Type III)

Trypticase peptone

Proteose peptone

Yeast Extract

KCl

NaCl

CaCl₂

Na₂HPO₄

NaHCO₃

Hemin

Menadione

Urea

L-Arginine

Equipment

1000 mL beaker

100 mL graduated cylinder

2 × 1000 mL bottle

Magnetic stirrer (with heating element)

Autoclave

pH measure



Protocol materials

☒ Bacto Yeast Extract **Becton Dickinson (BD)**

☒ Mucin from porcine stomach (Type III) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1778**

☒ Oxoid® Proteose Peptone **Thermo Fisher Catalog #LP0085B**

☒ Trypticase® Peptone **Thermo Fisher Catalog #211921**

☒ CaCl₂

☒ Hemin

☒ NaCl

☒ NaHCO₃

☒ KCl

☒ Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #7558-79-4**

☒ NaOH

☒ Menadione

☒ Urea

☒ L-Arginine **Catalog #A5006**

Before start

Mix the solution under a fumehood. It can smell pretty bad.

Also have the incubator under a fumehood, if possible. I had no such luxury...



1 Add  300 mL distilled (or deionized) dH₂O to a  1000 mL beaker, with stirring and heat  60 °C .

2 Add:

-  2.5 g



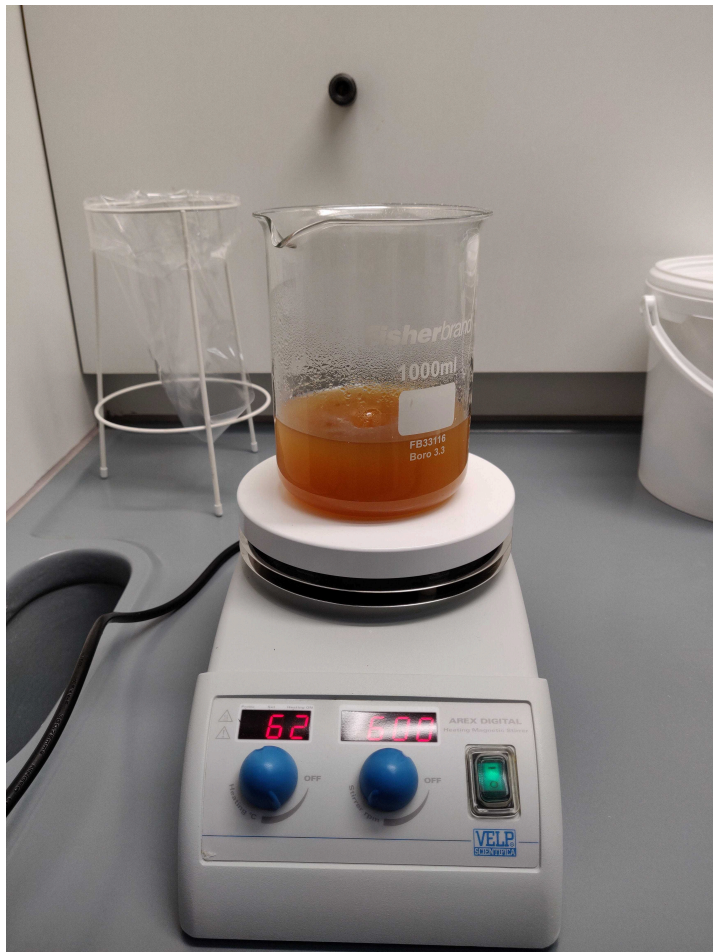
Mucin from porcine stomach (Type III) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1778**

-  5 g  Trypticase®; Peptone **Thermo Fisher Catalog #211921**

-  10 g  Oxoid®; Proteose Peptone **Thermo Fisher Catalog #LP0085B**

-  5 g  Bacto Yeast Extract **Becton Dickinson (BD)**

Let the reagents completely dissolve before continuing to the next step



3 Add:

-  2.5 g  KCl

-  0.35 g  NaCl

-  0.2 g  CaCl₂

-  0.74 g

 Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #7558-79-4**

-  0.54 g  NaHCO₃

-  2.5 mg  Hemin

- 4 Add the remaining  700 mL distilled (or deionized) H₂O



- 5 Adjust to  7 with  NaOH and stirring
- 6 Transfer to two 1000 ml bottles, so around half of each bottle is filled.

15m

Autoclave at  121 °C ,  1 Bar for  00:15:00 minutes

Safety information

Do NOT screw bottle caps on tightly before autoclaving.

Loosely screw the caps on the bottles or cover the tops with foil.



7 Once the solution has cooled, combine solutions to a single 1000ml bottle, and add:

-  1 mg  Menadione
-  0.3 g  Urea
-  0.17 g  L-Arginine **Catalog #A5006**

Safety information

These should be filter-sterilised as there are no more decontamination steps for the artificial saliva.

Use clean gloves and take precautions not to contaminate the artificial saliva with bacteria or starch.

8 Store in fridge at ca.  4 °C

Occasionally test the pH of the solution to ensure it stays around  7

Protocol references

Sissons, C. H., Cutress, T. W., Hoffman, M. P., & Wakefield, J. S. J. (1991). A Multi-station Dental Plaque Microcosm (Artificial Mouth) for the Study of Plaque Growth, Metabolism, pH, and Mineralization: Journal of Dental Research. <https://doi.org/10.1177/00220345910700110301>