VERSION 2

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

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Artificial saliva V.2

In 1 collection

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BYOC

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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
$CaCl_2$
Na_2HPO_4
$NaHCO_3$
Hemin
Menadione
Urea
L-Arginine

Equipment

1000 mL beaker
100 mL graduated cylinder
2 x 1000 mL bottle
Magnetic stirrer (with heating element)
Autoclave
pH measure

BEFORE START INSTRUCTIONS

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...

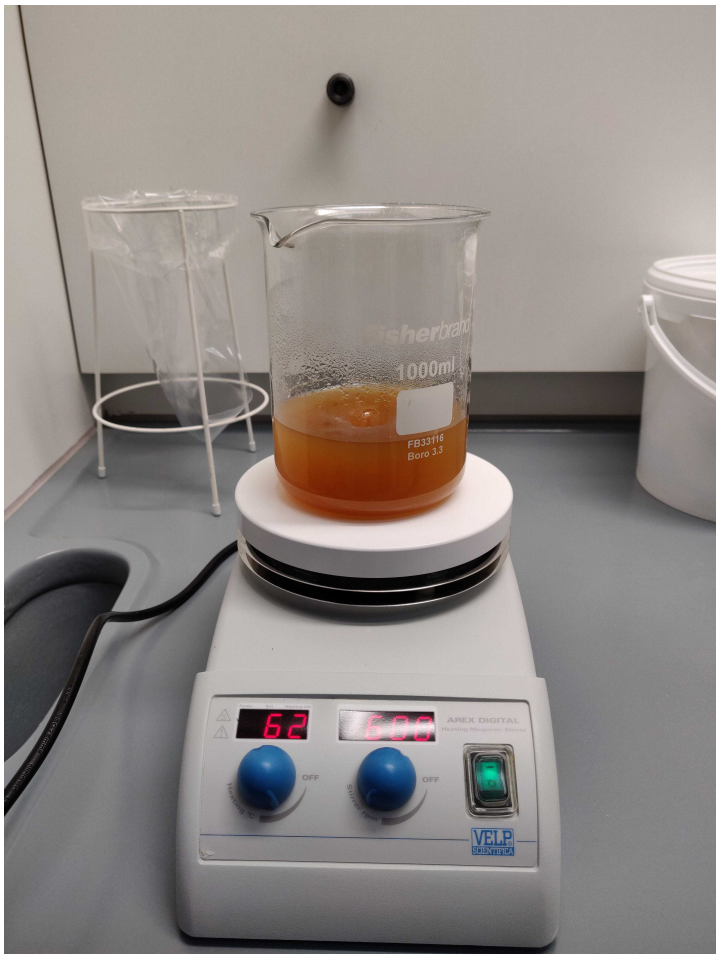
1 Add 300 mL distilled (or deionized) dH_2O to a 1000 mL beaker, with stirring and heat 60 °C .

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**

Let the reagents completely dissolve before continuing to the next step



3 Add:

- 2.5 g KCl **Contributed by users**
- 0.35 g NaCl **Contributed by users**
- 0.2 g CaCl2 **Contributed by users**
- 0.74 g Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #7558-79-4**
- 0.54 g NaHCO3 **Contributed by users**

- 2.5 mg Hemin **Contributed by users**

4 Add the remaining 700 mL distilled (or deionized) H_2O

5 Adjust to pH 7 with NaOH **Contributed by users** 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 **Contributed by users**
- 0.3 g Urea **Contributed by users**
- 0.17 g L-Arginine **Contributed by users 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 pH 7