Artificial saliva V.1

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Creating an artificial saliva solution for oral biofilm growth.

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

Bjorn Bartholdy, a.g.henry 2021. Artificial saliva. **protocols.io**
https://protocols.io/view/artificial-saliva-bva9n2h6

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In steps of
Amylase activity
Biofilm growth with starch treatment
Biofilm growth with starch treatment
Biofilm growth with starch treatment
Amylase activity
Biofilm growth with starch treatment

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

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
2 x 1000 mL bottle
Magnetic stirrer (with heating element)
Autoclave
pH measure

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:

- Mucin from porcine stomach (Type III) **Sigma**
  2.5 g **Aldrich Catalog #M1778**
- Trypticase™ Peptone **Thermo**
  5 g **Fisher Catalog #211921**
- Oxoid™ Proteose Peptone **Thermo**
  10 g **Fisher Catalog #LP0085B**
- Bacto Yeast Extract **Becton-Dickinson**
  5 g

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
- Sodium phosphate dibasic Sigma
- 0.74 g 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) dH_2O

5 Adjust to pH 7 with NaOH Contributed by users and stirring

6 Transfer to two 1000 ml bottles, so half of each bottle is filled. 15m

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

> Do NOT screw bottle caps on tightly.
>
> Loosely screw the caps on the bottles or cover the tops with foil

7 Once the solution has cooled, add:

- 1 mg Menadione Contributed by users
- 0.3 g Urea Contributed by users
- L-Arginine Contributed by users Catalog #A5006
- 0.17 g

8 Store in fridge at ca. 4 °C

Occasionally test the pH to ensure it stays around pH 7