1

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Amylase activity V.1

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protocol .

BYOC

Bjorn Bartholdy

This protocol is a scaled-down, modified version of the Enzymatic Assay of α-Amylase (EC 3.2.1.1) found here.

Bjorn Bartholdy, a.g.henry 2021. Amylase activity . **protocols.io** https://protocols.io/view/amylase-activity-bw8jphun

protocol ,

Aug 09, 2021

Oct 27, 2021

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Chemicals

Potato starch
D-(+)-maltose monohydrate
Sodium phosphate monobasic
Sodium chloride
potassium sodium tartrate, tetrahydrate
Sodium Hydroxide
3,5 Dinitrosalicylic acid

Equipment

Pipettes

- 2 – 20 µL
- 20 – 200 µL
- 100 – 1000 µL

8-channel pipette (ca. 20 - 300 µL) [OPTIONAL, but recommended]
96 well microplate
Spectrophotometer (with 540 nm filter)

All instances of dH_2O can be replaced with ultrapure water.

Prepare solutions 15m

15m

1 0.5% Potato starch solution

1. add a small amount of dH_2O to a beaker
2. add [M] **0.5 Mass Percent** 0.5% (w/v) potato starch
3. Boil for **00:15:00** while stirring
4. Take off heat and leave at room temperature
5. Add dH_2O to final volume
6. Continuous stirring throughout the assay

2 0.2% Maltose

1. add **100 mg** maltose to **50 mL** dH_2O

3 Buffer

1. add **250 mL** dH_2O to a beaker
2. add **600 mg** of sodium phosphate, monobasic
3. add **97.5 mg** of sodium chloride
4. adjust to pH **6.9** with 1 M NaOH **Contributed by users** and 1 M HCl **Contributed by users**

4 2 M NaOH

1. add **0.8 g** NaOH **Contributed by users** to **10 mL** dH_2O

5 5.3 M potassium sodium tartrate, tetrahydrate solution

1. add **14.96 mg** Potassium sodium tartrate tetrahydrate **Sigma Aldrich Catalog #S2377** to **10 mL** of the **2 M NaOH** solution

2. dissolve solids with heat and stirring

DO NOT heat to a boil

6 96 mM 3,5-Dinitrosalicylic acid solution

1. add **0.438 g** **3,5-dinitrosalicylic acid** to **20 mL** **dH_2O**
2. dissolve solids with heat and stirring

DO NOT heat to a boil

7 Colour reagent

1. add **12 mL** of **50-70 °C** **dH_2O** to an appropriate size amber bottle (or something that can protect the solution from light).
2. add (slowly and with mixing) **8 mL** of warm **5.3 Molarity (M)**
   Potassium sodium tartrate tetrahydrate **Sigma Aldrich Catalog #S2377**
3. add **20 mL** of warm **96 mM 3,5-Dinitrosalicylic acid solution**

The colour reagent is stable for 6 months in a dark place at ambient temperature

Saliva collection 30s

30s

8 Saliva samples are included as a positive control for amylase activity.

Saliva donors rinse their mouth with water for **00:00:30**

9 Collect the saliva by spitting into 50 ml plastic centrifuge tubes.

10 Centrifuge the saliva at **1000 x g, 00:10:00** and sample from the supernatant. 10m

Standard curve preparation

11 Two standard curves are prepared: one containing dH_2O, and one containing artificial saliva.

Artificial saliva
by Bjorn Bartholdy
PREVIEW RUN

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

11.2 Add:

- **2.5 g** Mucin from porcine stomach (Type III) **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

11.3 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**

11.4 Add the remaining **700 mL** distilled (or deionized) dH_2O

11.5 Adjust to **pH 7** with NaOH **Contributed by users** and stirring

11.6 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

11.7 Once the solution has cooled, add:

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

11.8 Store in fridge at ca. **4 °C**

Occasionally test the pH to ensure it stays around **pH 7**

Add the following reagents to the wells of a deep-well microplate so each well contains a total

12 volume of 225 µL. Each column in the table represents a single well.

Add the Maltose to each well first, then dH_2O, then colour reagent.

Reagent	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD Blank
0.2% Maltose	3.75	15	30	45	60	75	150	0
dH2O	146.25	135	120	105	90	75	0	150
Colour reagent	75	75	75	75	75	75	75	75

All quantities are in µL

13 **go to step #12** and repeat the process, but adding artificial saliva instead of dH_2O.

14 Boil the deep-well microplate(s) for **00:03:00** , then cool on ice to room temperature. 3m

15 Add **675 µL** of dH_2O to each well for a final volume of **900 µL** .

Sample preparation

16 Samples are prepared in a 96 deepwell plate with approx. 1 mL volume per well.

Samples should be analysed in duplicates or triplicates.

17 To each well, add **75 µL** of sample (or saliva)

It is best to make duplicates or triplicates of the samples

18 Then add **75 µL** of the **0.5% potato starch solution** to each of the wells with samples (and saliva)

Once the starch has been added to the sample, the reaction will begin. The starch

Once the starch has been added to the sample, the reaction will begin. The starch should be added quickly; ideally with a multichannel pipette .

19 Incubate the plate at **36 °C** for **00:06:00** 6m

20 Remove the plate from the incubator and add **75 µL** of colour reagent to the sample wells, then boil for **00:03:00** . 3m

The colour reagent will stop the reaction, so should be added with a multichannel pipette.

21 Cool the plate on ice to **Room temperature**

then add **675 µL** **dH_2O** to each well for a final volume of **900 µL** (homogenise the solution with the pipette).

Use a new pipette tip for each sample.

22 then transfer **200 µL** to a 96 well microplate suitable for the photometer.

Photometer

23

Multiskan FC
Microplate Photometer
Thermo Scientific **51119000**

Photometer settings:
540 nm filter

1. Photometer reading 1
2. Pause
3. Shake
4. Pause
5. Photometer reading 2

Make sure to include a sample blank (with dH_2O for saliva positive controls, and stock artificial saliva for samples)

Calculations

24 Calculate ΔA_{540} of each Standard by subtracting the Standard Blank.

$$\Delta A_{540\ Standard} = \Delta A_{540\ Standard} - \Delta A_{540\ StandardBlank}$$

25 Prepare the standard curve by regressing (OLS) the ΔA_{540} of each Standard on mg Maltose (go to step #12)

26 Calculate ΔA_{540} of each Sample by subtracting the Sample Blank.

$$\Delta A_{540\ Sample} = \Delta A_{540\ Sample} - \Delta A_{540\ SampleBlank}$$

27 Then calculate the mg of Maltose released (x) using the regression coefficients:

$$\frac{y-b}{a} = x$$

Where *y* is the $\Delta A^{540}\ Sample$, *b* is the intercept, and *a* is the slope.

28 Units per mL enzyme can be calculated as:

$$U/mL\ enzyme = \frac{mg\ Maltose\ released \times dilution\ factor}{mL\ enzyme}$$

where *dilution factor* is how much the sample was diluted (if at all), and *mL enzyme* is how much of the sample was added in step 17. **go to step #17**

A *Unit* is defined as mg of maltose released from starch in 6 minutes.