Megazyme

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ASSAY OF

AMYLOGLUCOSIDASE

using

p-NITROPHENYL β-MALTOSIDE

plus

Thermostable β-Glucosidase

R-AMGR3 08/18

NOTE:

The AMG assay reagent previously supplied by Megazyme contained *p*-nitrophenyl-β-maltoside (4 mM), plus **almond seed β-glucosidase** (25 U/mL). While this substrate worked well, its use was limited to a narrow pH and temperature range. Also, stability was limited by the purity of the almond β-glucosidase then available. Recently, this substrate has been improved by replacing almond β-glucosidase with a thermostable β-glucosidase. The advantages are:

1. The high purity of the thermostable β-glucosidase means that the substrate is stable for longer periods of time.

2. Because the enzyme is thermostable, the reagent can be used at temperatures up to 60°C (the preferred temperature for the assay of AMG).

However, because thermostable β-glucosidase is unstable at very low salt concentrations, salt and buffer are added to the dry reagent. When dissolved, the substrate solution will have a pH of ~ 6.0 (the optimal pH for stability of the β-glucosidase).

SUBSTRATE:

***p*-Nitrophenyl-β-maltoside (4 mM),**
plus thermostable β-glucosidase (5 U/mL).
Dissolve the contents of one vial in 10 mL of distilled water, divide into aliquots of 2-3 mL and store frozen. Store on ice during use.

BUFFER:

100 mM Sodium acetate buffer (pH 4.5).
Add 5.9 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH to pH 4.4 by addition of 1 M (4 g/100 mL) NaOH solution. Adjust the volume to 1 L and store in a well sealed bottle at 4°C.

SAMPLE PREPARATION:

Add 1 mL of enzyme preparation to 9 mL of 100 mM sodium acetate buffer (pH 4.5) and mix well. Repeat this dilution step until the enzyme is suitably diluted for assay.

ASSAY PROCEDURE:

1. Pre-equilibrate enzyme solution at 40°C for 5 min.
2. To 0.2 mL of pre-equlibrated substrate solution add 0.2 mL of suitably diluted (and pre-equilibrated) enzyme solution. Mix well and incubate at 40°C for exactly 10 min.

3. Terminate the reaction and develop the colour by adding 3.0 mL of 2% tris base buffer (pH ~ 8.5; Megazyme **B-TRIS500**).
4. Measure the absorbance at 400 nm against a reagent blank.

NOTE:
The reagent blank is prepared by adding 3.0 mL of Tris base buffer (2%) to 0.2 mL of reagent mixture with vigorous stirring, followed by the enzyme solution (0.2 mL) with stirring.

CALCULATION OF ACTIVITY:

$$\text{Activity (U/mL)} = \frac{\Delta A_{400}}{10} \times \frac{3.4}{0.2} \times \frac{1}{18.1} \times \text{Dilution}$$

where:

U = International units of enzyme activity. One Unit is the amount of enzyme which releases one µmole of *p*-nitrophenol from the substrate per minute at the defined pH and temperature

ΔA_{400} = Absorbance (reaction) - Absorbance (blank)

10 = Incubation time

3.4 = Final reaction volume (mL)

0.2 = Volume of enzyme assayed (mL)

18.1 = E_{mM} *p*-nitrophenol in 2% ris base (pH ~ 8.5) at 400 nm

The Units of amyloglucosidase activity obtained using the above assay, can be related to activity on maltose (10 mg/mL) or soluble starch (10 mg/mL) at 40°C and pH 4.5, using the following equations:

Enzyme Units on Maltose = 1.8 x Units on ***p*NP-β-maltoside.**

Enzyme Units on Starch = 11.5 x Units on ***p*NP-β-maltoside**.

REFERENCE:

McCleary, B. V., Bouhet, F. & Driguez, H. (1991). "Measurement of amyloglucosidase using *p*-nitrophenyl β-maltoside as substrate" *Biotechnology Techniques*, **5,** 255-258.

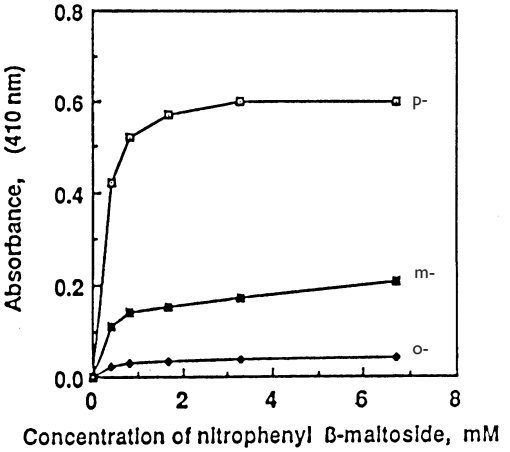

Figure 1. Optimisation of the concentration of nitrophenyl β-maltoside in the reagent mixture.
Substrates: ■, *m*-; ♦, *o*-; □, *p*-nitrophenyl β-maltosides

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