

Megazyme

ASSAY OF
AMYLOGLUCOSIDASE

using

**p-NITROPHENYL
β-MALTOSE**

plus

Thermostable β-Glucosidase

R-AMGR3 05/04



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:

200 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 (approx. 30 mL is required). 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 200 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-equilibrated 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% trizma base solution (pH ~ 8.5; Sigma T-1503).
4. Measure the absorbance at 400 nm against a reagent blank.

NOTE:

The reagent blank is prepared by adding 3.0 mL of Trizma base solution (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 μ mole 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% trizma 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 = 2.0 x Units on ***p*NP- β -maltoside**.

Enzyme Units on Starch = 15 x Units on ***p*NP- β -maltoside**.

REFERENCE:

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

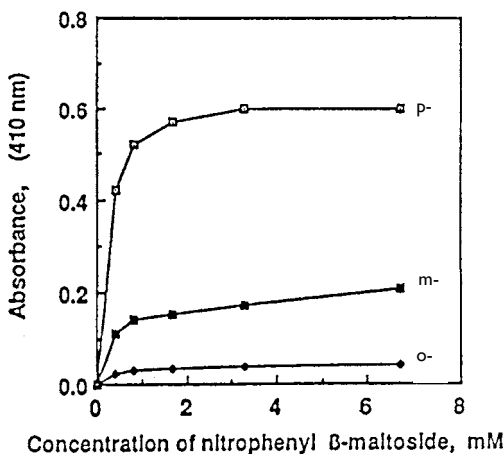


Figure 1. Optimisation of the concentration of nitrophenyl β -maltoside in the reagent mixture.
Substrates: ■, *m*-; ◆, *o*-; □, *p*-nitrophenyl β -maltosides



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