SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of LYTICASE

PRINCIPLE:

Yeast Cells (intact) $\xrightarrow{\text{Lyticase}}$ Yeast Cells (lysed)

CONDITIONS: T = 25°C, pH 7.5, A_{800nm}, Light path = 1 cm

METHOD: Turbidimetric Rate Determination

REAGENTS:

A. 67 mM Potassium Phosphate Buffer, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 25°C with 1 M KOH.)

B. 0.2% (w/v) Bakers Yeast Substrate Suspension (YSC-1)
(Prepare 100 ml in deionized water using Yeast, Saccharomyces cerevisiae, Bakers Yeast, Sigma Stock No. YSC-1. Allow solution to stir for 2 hours before use.)[1]

C. Lyticase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 500 units/ml[2] of Lyticase in cold deionized water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:[3]

	Test 1	Test 2	Test 3	Blank
Deionized Water	0.55	0.54	0.53	0.60
Reagent A (Buffer)	1.50	1.50	1.50	1.50
Reagent B (YSC-1)	0.90	0.90	0.90	0.90
Reagent C (Enzy Soln)	0.05	0.06	0.07	------

Mix the Tests and Blank samples by inversion.[4] Monitor the decrease in A_{800nm} for approximately 10 minutes. Obtain the ΔA_{800nm}/minute using the maximum linear rate for both the Tests and Blank.[5] The ΔA_{800nm} for the blank should cover only the range of the times chosen for each of the Test samples.[6]

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{800nm}/\text{min Test} - \Delta A_{800nm}/\text{min Blank})(df)}{(0.001)\ (0.1)}$$

df = Dilution factor
0.001 = Change in absorbance at 800 nm as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will produce a ΔA_{800nm} of 0.001 per minute at pH 7.5 at 25°C, using a suspension of yeast as substrate in a 3 ml reaction mix.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 34 mM potassium phosphate, 0.1% (w/v) baker's yeast, and 25,30, and 35 units lyticase.

REFERENCE:

Scott, J.H. and Schekman, R. (1980) *Journal of Bacteriology* **142**, 414-423

NOTES:

1. YSC-1 substrate solution was found to be stable for up to 8 hours after preparation.

2. Preparation of 500 units/ml is crucial to obtaining valid activity in this procedure. There is an inverse relationship between the enzyme concentration and the activity.

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NOTES: (continued)

3. The amount of enzyme added to each of the three cuvettes is critical as noted above in note 3. Thus, aliquots of 0.05, 0.06, and 0.07 ml should be followed.

4. It is important that both the test samples and the blank are inverted due to the settling of the suspended yeast in the reaction mixture.

5. It is crucial that a 2 minute time window be chosen in order to achieve consistent results. Any smaller time window may result in a falsely high rate due to settling of the substrate or other sources of noise in the assay. Additionally, any larger time window may gradually decrease the true maximum rate of the enzyme. Also, the ΔA_{800nm}/min must fall between 0.025 and 0.035 in order for the assay to be valid.

6. The blank is an important measurement due to the settling of the substrate in the cuvettes over time and its potential to create a false rate. Thus, if the maximized ΔA_{800nm}/min of the various enzyme levels are not in the exact same time window, each level needs to be coupled with separately calculated ΔA_{800nm}/min for the blank.

7. This assay is based on the cited references.

8. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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