SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of CELLULASE

(EC 3.2.1.4)

PRINCIPLE:

Cellulose + H_2O $\xrightarrow{\text{Cellulase}}$ D-Glucose

D-Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ D-Glucose 6-Phosphate + ADP

D-Glucose 6-Phosphate + β-NAD $\xrightarrow{\text{G-6PDH}}$ 6-PG + β-NADH

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
G-6PDH = Glucose 6-Phosphate Dehydrogenase
G-PG = 6-Phospho-D-Gluconate

CONDITIONS: T = 37°C, pH = 5.0, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 200 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)

B. 5% (w/v) Sigmacell Solution (Sigmacell)
(Prepare 100 ml in Reagent A using Cellulose (Sigmacell), Type 20, Sigma Prod. No. S-3504. Mix and heat gently to make a uniform suspension.)

C. Cellulase Enzyme Solution (Cellulase)
(Immediately before use, prepare a solution containing 2 - 6 units/ml of Cellulase in cold deionized water.)

D. Glucose (HK) Determination Vial (16-10)
(Use Glucose (HK) 10, Sigma Stock No. 16-10. Dissolve the contents in 10 ml of deionized water.)

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PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into suitable tubes:

	Test	Blank
Reagent B (Sigmacell)	4.00	4.00

Equilibrate to 37°C. Then add:

Reagent C (Cellulase)	1.00	------
Deionized Water	------	1.00

Immediately mix by swirling and incubate at 37°C for exactly 120 minutes with moderate shaking.

Immediately transfer into an ice bath. Allow to stand until the suspension is settled. Centrifuge for 2 minutes to clarify. Use the supernatant in Step 2.

Step 2:

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

	Test	Blank
Reagent D (16-10)	3.00	3.00

Equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both the Test and Blank. Then add:

Test Supernatant (Step 1)	0.10	------
Blank Supernatant (Step 1)	------	0.10

Immediately mix by inversion and record the increase in A_{340nm} until complete (for approximately 5 minutes). Obtain the final A_{340nm} for both the Test and Blank.

CALCULATIONS:

ΔA_{340nm} Test = A_{340nm} Test Final - A_{340nm} Test Initial

ΔA_{340nm} Blank = A_{340nm} Blank Final - A_{340nm} Blank Initial

CALCULATIONS: (continued)

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}\ \text{Test} - \Delta A_{340nm}\ \text{Blank})(3.1)(5)(df)}{(6.22)(2)(1)(0.1)}$$

3.1 = Final volume (in milliliters) of Step 2
5 = Total volume (in milliliters) of reaction mix (Step 1)
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340nm
2 = Conversion factor from 2 hours to 1 hour as per the Unit Definition
1 = Volume (in milliliter) of cellulase used in Step 1
0.1 = Volume (in milliliter) from Step 1 used in Step 2

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

UNIT DEFINITION:

One unit will liberate 1.0 µmole of glucose from cellulose in one hour at pH 5.0 at 37◦C (2 hour incubation time).

FINAL ASSAY CONCENTRATION:

In a 5.00 ml reaction mix, the final concentrations are 40 mM sodium acetate, 4% (w/v) Sigmacell and 2 - 6 units of cellulase.

REFERENCE:

Worthington, C.E. (1988) *Worthington Enzyme Manual*, pp. 76-79, Worthington Biochemical Corporation, Freehold, NJ

NOTES:

1. This assay is based on the cited reference.

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