SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of CARBONIC ANHYDRASE
(EC 4.2.1.1)

PRINCIPLE:

$CO_2 + H_2O \xrightarrow{\text{Carbonic Anhydrase}} H_2CO_3$

CONDITIONS: T = 0°C, pH 8.3

METHODS: Titrimetric

REAGENTS:

A. 20 mM Tris Sulfate Buffer, pH 8.3 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.3 at 25°C with 2 N H_2SO_4. Store buffer on ice.)

B. CO_2 Saturated H_2O Solution (Substrate Solution)
(Prepare 200 ml in deionized water by bubbling CO_2 (dry ice in Erlenmeyer flask) through the H_2O for 30 minutes. Perform this step on ice. Wait 20 minutes before using.)

C. Carbonic Anhydrase Enzyme Solution
(Prepare a solution containing 1 mg/ml of solid Carbonic Anhydrase in cold deionized water. Immediately prior to use, dilute the enzyme to 10 - 60 units/ml with cold deionized water.)

PROCEDURE:

Immediately prior to performing the assay on the blank, pipette (in milliliters) the following reagents into a 4 dram vial which is on ice.

	Blank
Reagent A (Buffer)	3.00
Deionized Water	0.05
Reagent B (Substrate)	2.00

PROCEDURE: (continued)

Immediately after the addition of Reagent B (Substrate), insert the probe of a standardized pH meter into the Blank vial. Record the time (T) required for the pH to change from 8.3 to 6.3, or if the pH drops below 8.3 before the pH probe is placed in reaction mixture. Begin recording the time upon addition of water.

Continue repeating the Blank measurements until at least three values of T are within a 15 second time window and the average of these three values is $T_{Blank,avg}$ between 70-100 seconds.[1]

Immediately prior to performing the assay on the Test, pipette (in milliliters) the following reagents into a 4 dram vial which is on ice.

	Test
Reagent A (Buffer)	3.00
Reagent C (Enzyme)	0.05
Reagent B (Substrate)	2.00

Immediately after the addition of Reagent B (Substrate), insert the probe of a standardized pH meter into the test vials. Record the time (T) required for the pH to change from 8.3 to 6.3. Perform in triplicate.[2] If the pH drops below 8.3 before the pH probe is placed in the reaction mixture, begin recording time immediately upon addition of enzyme.

Repeat the Blank measurement after running the test vials. If the Blank is more than 20% from ($T_{Blank,avg}$), Reagent B (substrate) may have deteriorated and the assay must be repeated.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(T_{Blank,avg} - T_{Test,avg})(df)}{(T_{Test,avg})(0.05)}$$

df = Dilution factor
T = Time (in seconds) required for the pH to change from 8.3 to 6.3 as per the Unit Definition
0.05 = Volume (in milliliter) of enzyme used

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

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One Wilbur-Anderson (W-A) unit will cause the pH of a 0.02 M Trizma buffer to drop from 8.3 to 6.3 per minute at 0°C. (One W-A unit is essentially equivalent to one Roughton-Booth unit.)

FINAL ASSAY CONCENTRATION:

In a 5.05 reaction mix, the final concentrations are 12 mM Tris and 0.5 - 3 units carbonic anhydrase.

REFERENCES:

Wilbur, K.M. and Anderson, N.G. (1948) *Journal of Biological Chemistry* **176**, 147-154

Worthington, C.C. (1988) in *Worthington Enzyme Manual* (Worthington, C.C. ed.) pp 57-59, Worthington Biochemical Corporation, Freehold, NJ

NOTES:

1. Because assay results are somewhat dependent on Blank rates, Sigma has standardized this assay so that the Blank must be between 70 - 100 seconds. If the Blank is too fast, swirl or stir Reagent B (Substrate Solution) to remove dissolved CO_2. If the Blank is too slow, continue to bubble CO_2 through water and then allow to equilibrate.

2. If test times are less than 20 seconds, the enzyme must be further diluted and the test repeated.

3. This assay is based on the cited references.

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