

SIGMA[®]**CARBON DIOXIDE ASSAY KIT****Kit NO. CDA-20**

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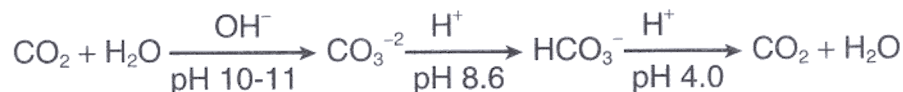
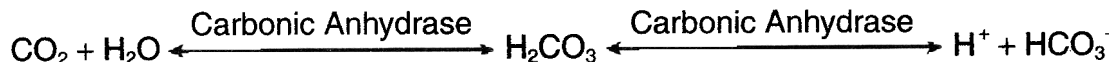
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Internet: <http://www.sigma.sial.com>**INTRODUCTION**

Enzymes, as analytical tools, have found widespread use in the food, biochemical and pharmaceutical industry. Enzymatic methods are specific, reproducible, sensitive, rapid and therefore ideal for analytical purposes. Due to the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.

This kit is for the enzymatic, titrimetric determination of carbon dioxide.

PRINCIPLE

The pH of the sample solution is adjusted to pH 10 - 11 with NaOH. Carbonic Anhydrase is added and the solution is titrated with sulfuric acid to pH 8.6 where essentially all of the carbon dioxide exists as bicarbonate ion. The bicarbonate ion is titrated with sulfuric acid to pH 4.0 where essentially all of the bicarbonate ion becomes carbon dioxide. The titer measured between pH 8.6 and 4.0 is equal to the molar amount of dissolved CO₂.

Carbonic Anhydrase catalyzes the reaction of carbon dioxide to carbonic acid and improves the overall accuracy of the analysis by preventing premature loss of carbon dioxide at intermediate pH's.

KIT REAGENTS

Refer to Material Safety Data Sheets for updated risk, hazard or safety information.

1. Carbonic Anhydrase for Carbon Dioxide Assay Kit (Sigma Product No. C 9207)

Reconstitute reagent vial with 3.5 ml of deionized water. Stopper vial and mix several times by inversion. DO NOT SHAKE.

Each vial, when reconstituted with 3.5 ml of deionized water, contains approximately 0.1 mg protein/ml of Carbonic Anhydrase (Bovine Erythrocytes).

The dry reagent is stored at -20°C . Reconstituted reagent is stable, in the absence of visible microbial growth, for 7 days at $2-8^{\circ}\text{C}$.

2. Sodium Carbonate, Standard for Carbon Dioxide Assay Kit (Sigma Product No. C 9082)

Used as a control to ensure assay reliability. Dry reagent is stable for at least 2 years when stored desiccated at room temperature.

REAGENTS NOT PROVIDED IN KIT

1. Sulfuric Acid (Sigma Product No. S 1526)

Prepare a 0.0455 N H_2SO_4 solution.

2. Sodium Hydroxide (Sigma Product No. S 0899)

Prepare a 50% (w/v) solution.

APPARATUS

1. pH meter, standardized at pH 4 and 10 with appropriate buffers.

2. Buret, 25 ml.

3. Magnetic stirrer.

DETERMINATION

This method is applicable to ≤ 400 mg CO₂/100 ml

TEST

1. Cool sample in closed container to 5°C.
2. Open container and add 5 ml of 50% NaOH per 375 ml of sample (or an amount of 50% NaOH that will give a pH between 10 and 11). Immediately close container and mix contents.
3. Pipet 10 ml of sample solution from #2 into a 150 ml beaker containing 40 ml of deionized water. Add 150 μ l of Carbonic Anhydrase solution.
4. Titrate solution to pH 8.6 with 0.0455 N H₂SO₄. Refill buret.
5. Titrate solution to pH 4.0 with 0.0455N H₂SO₄. Record volume of H₂SO₄ used to titrate between pH 8.6 and 4.0.

BLANK

1. Combine 25 ml of sample and 150 μ l of Carbonic Anhydrase solution in a 500 ml filter flask.
2. Degas solution for 1 minute with shaking under vacuum.
3. Add 0.33 ml of 50% NaOH to solution from #2 (or a volume equal to 1/15 of the 50% NaOH that was added to the test sample).
4. Pipet 10 ml of solution from #3 into a 150 ml beaker containing 40 ml of deionized water.
5. Titrate solution to pH 8.6 with 0.0455 N H₂SO₄. Refill buret.
6. Titrate solution to pH 4.0 with 0.0455N H₂SO₄. Record volume of H₂SO₄ used to titrate between pH 8.6 and 4.0.

CALCULATIONS

$$\begin{aligned}\text{mg CO}_2/100 \text{ ml} &= [\text{ml Titrant-Sample} - \text{ml of Titrant-Blank}] \times \text{N of H}_2\text{SO}_4 \times 44 \times 10 \times F \\ &= [\text{ml Titrant-Sample} - \text{ml of Titrant-Blank}] \times 0.0455 \times 44 \times 10 \times 1.013 \\ &= [\text{ml Titrant-Sample} - \text{ml of Titrant-Blank}] \times 20.28\end{aligned}$$

Where: Normality of H₂SO₄ = 0.0445 N

Molecular Weight of Carbon Dioxide = 44

10 = 100 ml of sample in equation / 10 ml of sample in assay

F = Assay Dilution Factor = (375 ml sample + 5 ml NaOH) \div 375 ml sample = 1.013

REFERENCES

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