

Carbon Dioxide Detection Assay

Catalog Number: EZ2014-01

For Research Use Only. Not for use in Diagnostic Procedures.

1. Intended Use

For plate-based colorimetric enzymatic determination of carbon dioxide content in environmental samples. The kit uses a spectrophotometric assay to detect carbon dioxide directly from samples. The unique features of the kit are:

- High sensitivity
- Rapid
- Robust
- High reproducibility
- Flexible format
- **Linearity:** 0-40mmol/L
- **Sensitivity:** Based on an instrument resolution of Abs. = 0.001, this procedure has a sensitivity of 0.03 mmol/L

2. Introduction

For plate-based colorimetric enzymatic determination of carbon dioxide content in environmental samples

The determination of CO_2 is an important marker in the environment. Elevated levels of atmospheric CO_2 are strongly associated with global temperature rise and water acidification. This kit is a simple, direct, and automation-compatible method for measuring sample CO_2 levels in samples. The assay uses a coupled enzyme assay to detect CO_2 (as HCO_3^-) as follows.

In the first step, the bicarbonate condenses with phosphoenolpyruvate to form oxaloacetate (and phosphate); this reaction is catalyzed by the enzyme phosphoenolpyruvate carboxylase (PEPC). The oxaloacetate is then reduced by malate dehydrogenase (MDH), using an NADH cofactor, to form malate and NAD+.

$$\text{Phosphoenolpyruvate} + HCO_3^- \xrightarrow{\text{PEPC}} \text{Oxaloacetate} + HPO_4^{2-}$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

Since the NADH molecule absorbs light at 340 nm but the NAD+ does not, the decrease in absorbance is dependent on the presence of CO_2 in the reaction. Therefore, the CO_2 analyte causes a decrease in absorbance at 340 nm that is directly proportional to the CO_2 concentration in the samples. The kit comes with a CO_2 standard that contains 30 mmol/L of sodium bicarbonate in an aqueous solution. The kit contains sufficient materials to rapidly test 42 samples in duplicate.

3. Procedure Overview

After preparing and diluting the sample, the assay is performed by adding Carbon Dioxide Reagent into microplate wells containing 3µL diluted sample. After 5 minutes incubation at 37°C, the absorbance of each well at 340nm is then measured using a plate reader. The concentration of carbon dioxide in each sample is then directly determined from the 340nm absorbance.

This kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). Store the kit at 4°C. The shelf life is 6 months after receipt when the kit is properly stored.

4. Kit Contents (96 determinations)

Component Name	Volumes	Storage
Carbon Dioxide Standard (30 mmol/L)	1 mL	2-8°C
CO_2-free Water	2 x 25 mL	2-8°C
Carbon Dioxide Reagent	4 vials	2-8°C
Microtiter Plate	1 each	4-25°C

5. User Supplied Materials

- Micro-pipettes with disposable plastic tips to pipet 5-20 µL.
- Micro-pipettes with disposable plastic tips to pipet 20-200 µL.
- Timer
- Microtiter plate reader (wavelength 340 nm)

6. Precautions

Add standards to plate only in order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve.

1. Carefully prepare at least 20 µL of sample using standard production procedure (if determinations are performed in singlet, then 3 µL is sufficient). Avoid contamination of reagents with CO_2. Do not blow into pipette. Do not open tubes unnecessarily. Keep container tightly stoppered.

2. Carefully dilute all samples with an equal volume of CO_2-free water. The diluted samples are now ready for use in the assay.

Note:

Samples with values above 40mmol/L should be further diluted with CO_2-free water and re-tested.

7. Instructions

Setup: Turn on the plate reader and allow lamp to warm up. Set the wavelength of the plate reader to 340nm and the temperature to 37°C.

Reagent Preparation: To reconstitute the Carbon Dioxide Reagent, add exactly 6 mL of CO_2-free water to the Carbon Dioxide Reagent. Mix by inversion 5-6 times. Avoid contamination of reagents with CO_2. Do not shake. Do not blow into pipette. Do not open tubes unnecessarily. Keep container tightly stoppered.

Reconstituted reagents stored in the closed, tightly stoppered, bottle are stable for 1 day at room temperature (18 -26° C) and 7 days refrigerated (2 -8° C).

IMPORTANT: The reconstituted Reagent can be left at room temperature for short periods (30 –60 minutes) prior to use. Between uses, keep the reconstituted Reagent in the closed tightly stoppered bottle at 4°C (for up to 7 days). Discard the Reconstituted reagent 7 days after reconstitution. This kit contains 4 vials of Carbon Dioxide Reagent; prepare another Carbon Dioxide Reagent if the previous one expires.

Preparation of Carbon Dioxide Standard Dilutions for Standard Curve:

- Label 6 microfuge tubes: 1, 2, 3, 4, 5, 6.
- Dilute the Carbon Dioxide Standard using CO_2-free water as described in the table below.
- After dilution, briefly mix each tube.

Tube #	Volume Carbon Dioxide Standard (30mmol/L)	Volume CO_2 free water	Carbon Dioxide Concentration (mmol/L)
1 (blank)	0ul	60ul	0
2	20ul	40ul	10
3	30ul	30ul	15
4	40ul	20ul	20
5	50ul	10ul	25
6	60ul	0ul	30

8. Sample Test Procedure

1. Pre-warm Reconstituted Carbon Dioxide Reagent at 37°C for 15 minutes.
2. Add 60 µl CO_2-free water to each standard tube. The standards and samples are now all diluted 1:1.
3. Add 3 µL of each diluted standard or diluted sample (in duplicate) to the microplate wells.
4. Add 300 µL of Carbon Dioxide Reagent to the wells.
5. Incubate at 37°C for 5 minutes.
6. Measure the absorbance of each well at 340 nm.
7. For each standard or sample, subtract the absorbance of each well from the absorbance of the blank (= A_{blank}, from Tube #1) to obtain the corrected absorbance for each point (= A_{blank}-$A_{standard}$ or = A_{blank}-A_{sample}) for the standards or the samples, respectively.

Carbon Dioxide Concentration Calculation:

There is a linear relationship between the concentration of CO_2 in a sample and corrected absorbance at 340 nm after a 5-minute incubation (A_{blank}-$A_{standard}$). Therefore, a standard curve used to calculate the CO_2 concentration in samples can be constructed by plotting the mean corrected absorbance values for the change of each of the diluted Carbon Dioxide standards (A_{blank}-$A_{standard}$) as a function of CO_2 concentration. The CO_2 concentration in samples can be determined using this standard curve.

9. Example Microplate Layout:

	A	B	C	D	E
1	Neg				
2	Neg				
3	Pos				
4	Pos				
5	Sample 1				
6	Sample 1				
7	Sample 2				
8	Sample 2				

10. Determination of CO_2 in Samples

If the enzyme activity in the sample is 20% lower than the negative control it is indicative that the sample may contain an CO_2 at the concentration above the limit of detection.

Notes on the measurement: The color of the reaction may continue to change after the specified reaction time has elapsed. The rate of the reaction is impacted by the room temperature thus, incubating the plate in a set temperature incubator at 25°C, it can help ensure consistency.

Note: If the test shows the concentration may actually be higher than a diluted standard. In this case, we recommend carrying out a stepwise dilution of the sample with distilled water, to bring the CO_2 content into the measuring range of the known concentration positive control. The dilution factor must be taken into account when calculating the CO_2 nt.

NOTE: FOR INFORMATION ON SAMPLE PREPARATION METHODS, CONTACT ATTOGENE AT SALES@ATTOGENE.COM FOR DETAILED INSTRUCTIONS.

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Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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