

Technical Bulletin

Boron Assay Kit

Catalog Number MAK448

Product Description

Boron is an essential micronutrient in plants and is involved in maintaining robust cell walls, cell membranes, and reproductive tissues. Although boron is common in the soil in its natural state as a borate mineral, the amount of boron available to plants is actually quite small. As a result, boron deficiency is the second most common micronutrient deficiency among crop plants. In order to keep plant boron levels in a healthy range, supplementation to the soil via fertilizers and additives is often required. If not regulated, a lack of or excess of boron may significantly lower crop yield. In the biotech industry, sodium borohydride is commonly used to conjugate antibodies and typically needs to be removed from the final product, especially for therapeutic antibodies.

The Boron Assay Kit provides a convenient and reliable means to measure boron. In the assay, borate complexes with azomethine-H to create a colored compound that can be measured at 420 nm. This assay can be used with a variety of samples and is simple, sensitive, and adaptable to high-throughput screening applications. The linear detection range of the assay is 0.05 to 10 µg boron/mL (0.05-10 ppm) when evaluating a 100 µL sample.

The kit is suitable for the quantitative determination of boron in water, plant tissue, soil samples, and antibody conjugation solutions.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

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|------------------------------|--------|
| • Reagent A | 250 mg |
| Catalog Number MAK448A | |
| • Reagent B | 20 mL |
| Catalog Number MAK448B | |
| • Standard (500 µg/mL Boron) | 150 µL |
| Catalog Number MAK448C | |

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Microcentrifuge capable of $RCF \geq 14,000 \times g$
- Hydrochloric Acid Solution, 1.0 N (Catalog Number H9892 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped ambient. Store components at 2-8 °C.

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Preparation Instructions

Briefly centrifuge Standard vial prior to opening.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Samples should be transparent and precipitate-free. If samples are cloudy or have precipitates, centrifuge for 5 minutes at $14,000 \times g$ and use clear supernatant for the assay.

Soil Samples

1. For dry weight analysis, first completely dry the soil to remove all water weight.
2. Mix 5 g of soil (sieved to remove rocks and debris) with 50 mL of 50 mM HCl for 30 minutes.
3. Centrifuge the sample to pellet precipitates and use the supernatant for the assay.

Note: If there are soil particles floating, avoid them when pipetting; the sample should be completely free of particulate matter.

Plant Samples

1. For dry weight analysis, first completely dry the plant matter to remove all water weight.
2. Burn 5 to 10 g of plant matter to ashes in a crucible at 500 °F for 3 hours in a drying oven. A blow torch can alternatively be used to burn to ashes if a drying oven is not available.
3. Mix the ashed plant material with 50 mL of 50 mM HCl for 30 minutes.

4. Centrifuge the sample to pellet the ash and use the supernatant for the assay.

Note: If there are ash particles floating, avoid them when pipetting; the sample should be completely free of particulate matter.

All samples

1. Transfer 100 μL of each Sample into two separate wells: one Sample well and one Sample Background well.
2. Add 150 μL of purified water to all Sample Background wells.
3. Add 250 μL of purified water to a separate well on the plate (Water Blank).

Standard Curve Preparation

1. Prepare a 10 $\mu\text{g/mL}$ Boron Standard by mixing 10 μL of the 500 $\mu\text{g/mL}$ Boron Standard with 490 μL of purified water.
2. Prepare Boron Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Boron Standards

Well	10 $\mu\text{g/mL}$ Boron Standard	Purified Water	Boron ($\mu\text{g/mL}$)
1	150 μL	-	10
2	90 μL	60 μL	6
3	45 μL	105 μL	3
4	-	150 μL	0

3. Mix well and transfer 100 μL of each Standard into separate wells of the plate.

Working Reagent

Note: The Working Reagent should be prepared fresh for each assay run and used within 15 minutes of preparation.

1. Mix enough reagents for the number of assays to be performed. For each Sample and Standard well, prepare 160 μL of Working Reagent according to Table 1. Mix well to dissolve Reagent A.



Table 1.

Preparation of Working Reagent

Reagent	Working Reagent
Reagent A powder	2 mg
Reagent B	160 µL

2. Add 150 µL of Working Reagent to each Standard and Sample well. Tap plate lightly to ensure the contents of the wells are mixed evenly.

Measurement

1. Incubate the plate for 40 minutes at room temperature, protected from light.
2. Read optical density (OD) at 420 nm.

Results

1. Subtract the OD_{Blank} (Standard #4) reading from the remaining Standard OD readings. Plot the corrected Standard OD readings against the Standard concentrations.
2. Determine the slope of the Standard curve using linear regression.
3. Calculate the Boron concentration of the sample:

Boron (µg/mL) =

$$\frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - (OD_{\text{SampleBG}} - OD_{\text{H}_2\text{O}}) \times DF}{\text{Slope (}\mu\text{g/mL}^{-1}\text{)}}$$

where

OD_{Sample} = OD value at 420 nm of SampleOD_{Blank} = OD value at 420 nm of Blank (Standard #4)OD_{SampleBG} = OD value at 420 nm of Sample BackgroundOD_{H₂O} = OD value at 420 nm of Water Blank Well

Slope = Slope of the Standard Curve

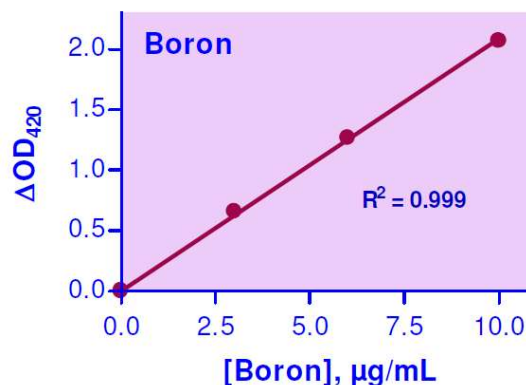
DF = Sample Dilution Factor (DF = 1 for undiluted samples)

If the calculated boron concentration of a sample is higher than 10 µg/mL, dilute the sample in purified water and repeat the assay. Multiply the result by the dilution factor (DF).

Conversions: 1 µg/mL of Boron equals 1 ppm or 92.5 µM.

Figure 1.

Typical Boron Standard Curve

**References**

1. Ziaeyan, A.H., and Rajaie, M., Combined effect of zinc and boron on yield and nutrients accumulation in corn. *Int. J. Plant Prod.*, **3(3)**, 35-44 (2012).
2. Uluisik, I., et al., The importance of boron in biological systems. *J. Trace Elem. Med. Biol.*, **45**, 156-162 (2018).
3. Shireen, F., et al., Boron: functions and approaches to enhance its availability in plants for sustainable agriculture. *Int. J. Mol. Sci.*, **19(7)**, 1856 (2018).

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