Technical Assistance
For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety
Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

General Limited Warranty

Zeatin Riboside Plate Kit Product Brochure
Catalog No. 20-0237
Rev. A

Intended Use
The Beacon Zeatin Riboside Plate Kit is an immunoassay for the detection of Zeatin Riboside in seaweed samples. This product is intended for research use only.

Principles
Zeatin Riboside HRP Enzyme Conjugate is pipetted into the test wells followed by the Calibrators and the Sample Extract(s). A soluble polyclonal Zeatin Riboside antibody solution is then added to the test wells to initiate the reaction. During an incubation, Zeatin Riboside and Zeatin Riboside HRP Enzyme Conjugate compete for binding to the soluble Zeatin Riboside antibody which is in turn immobilized on the test wells. Following the incubation, the wells are washed to remove any unbound Zeatin Riboside and Zeatin Riboside HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Zeatin Riboside concentration of the sample is derived.

Reagents and Materials Provided
1 Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
4 Vials of Zeatin Riboside Calibrators (0, 0.3, 1.5, and 10 ppb).
1 Bottle of Zeatin Riboside HRP Enzyme Conjugate.
1 Bottle of Zeatin Riboside Antibody.
1 Bottle of 10X Wash Concentrate (dilute prior to use).
1 Bottle of Substrate.
1 Bottle of Stop Solution.

Reagents and Materials Required but Not Provided
- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Materials for 1X wash solution preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (62°C to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Antibody, Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Zeatin Riboside Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Zeatin Riboside Plate Kits with different lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Sample Buffer Preparation (10% Methanol/PBS)

1. Measure 90 mL of Phosphate Buffered Saline (PBS) (pH 7.4) for each 100 mL being prepared and transfer to a clean glass container with a tight-fitting lid.
2. Measure 10 mL of methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix. Store tightly sealed to minimize evaporative loss.

Sample Preparation

Seaweed: (Dilution Factor: 100)

1. Weigh 0.5 g of the sample into a conical tube.
2. Measure 5 mL of Sample Buffer and add to the tube.
3. Vortex to thoroughly mix.
4. Transfer 1 mL of the extract to a microcentrifuge tube.
5. Centrifuge for 5 minutes at 2,000 x g.
6. Dilute the supernatant 1:10 in Sample Buffer and use in the assay.

1X Wash Solution Preparation

1. Measure 450 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Wash Concentrate bottle to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **50 µL of Enzyme Conjugate** into each well.
4. Dispense **50 µL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 µL of Antibody** into each well.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Solution and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
8. Dispense **100 µL of Substrate** into each well.
9. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
10. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

Result Interpretation

Semi-Quantitative Interpretation: Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrators:

- Samples with a lower absorbance (less color) than a calibrator have a concentration of Zeatin Riboside greater than the concentration of the calibrator.
- Samples with a higher absorbance (more color) than a calibrator have a concentration less than the concentration of the calibrator.

Quantitative Interpretation: It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

- The concentration of Zeatin Riboside in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- Samples with absorbances lower than the highest calibrator contain a concentration of Zeatin Riboside too high for quantification. Further dilute the sample extract in Sample Buffer to fit into the standard curve and retest along with the calibrators. Results must then be multiplied by the dilution factor used.
- Samples with Zeatin Riboside absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.3 ppb or > 10 ppb, respectively.