



Brevetoxin Plate Kit

Cat. # 20-0200

Instructional Brochure

READ COMPLETELY BEFORE USE

INTENDED USE

The Beacon Brevetoxin Plate Kit is a competitive ELISA for the quantitative analysis of brevetoxin in samples.

USE PRINCIPLES

The Beacon Brevetoxin Plate Kit is a competitive enzyme-labeled immunoassay. The brevetoxin HRP conjugate, sample extract, and calibrators are pipetted into the test wells followed by the brevetoxin antibody to initiate the reaction. During a 30 minute incubation period, brevetoxin from the sample and brevetoxin HRP conjugate compete for binding to the brevetoxin antibody. The brevetoxin antibody is captured onto the walls of the test wells. Following this 30 minute incubation, the contents of the wells are removed, and the wells are washed to remove any unbound brevetoxin, brevetoxin HRP conjugate and free brevetoxin antibody. After washing, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 30 minute incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown sample is compared to the color of the calibrators in the standard curve and the brevetoxin concentration of the sample is derived. The color intensity is inversely proportional to the amount of brevetoxin present.

MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8°C.

- 1 Plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with a desiccant
- 1 Vial each of 0.0 ppb, 0.1 ppb, 0.25 ppb, 0.5 ppb, 1.0 ppb and 2.5 ppb Brevetoxin (PBTX-3) Calibrator
- 1 Vial of Brevetoxin HRP Enzyme Conjugate
- 1 Vial of Polyclonal anti-Brevetoxin Antibody
- 1 Vial of Substrate
- 1 Vial of Stop Solution (Caution! 1N HCl. Handle with care.)
- 1 Instructional Brochure

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water
- Pipette with disposable tips capable of dispensing 50 µL and 100 µL
- Multi-channel pipette; 8 channels capable of dispensing 50 µL and 100 µL (optional)
- Paper towels or equivalent absorbent material
- Microwell plate or strip reader with 450 nm filter
- Timer
- Wash bottle

PERFORMANCE CHARACTERISTICS

Specificity

The following table shows the % cross reactivity of brevetoxin against other shellfish toxins.

Compound	% CR
Brevetoxin PBTX-3	100
PBTX-2	162
Neo Saxitoxin	< 1
Okadaic Acid	< 1
Saxitoxin	< 1

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Each reagent is optimized for use in the Beacon Brevetoxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Brevetoxin Plate Kits with different lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Do not use reagents after expiration date.
- Reagents should be brought to room temperature, 20°C to 28°C (62°F to 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- If running more than two strips at once, the use of a multichannel pipette is recommended for the addition of Antibody, Substrate and Stop Solution.
- Brevetoxin is a toxin and should be treated with care.
- The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal unused wells in the zip-lock bag with the desiccant.
3. Using a pipette with disposable tips, add **50 µL Enzyme Conjugate** into the appropriate test wells.
4. Dispense **50 µL of Calibrators or Sample Extract** into each well. Be sure to use a clean pipette tip for each.
5. Dispense **50 µL of Antibody Solution** into each test well.
6. Shake the plate gently for 30 seconds and incubate the test wells at room temperature for **30 minutes**.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory grade water and decant. Repeat 4 times for a total of five washes.
8. Following the last wash, tap the inverted wells onto an absorbent paper to remove the last of the wash solution.
9. Dispense **100 µL of Substrate** into each well.
10. Incubate the wells at room temperature for **30 minutes**.
11. Dispense **100 µL of Stop Solution** into each test well. Caution! 1N HCl. Handle with care.
12. Read and record the absorbance of the wells at 450 nm using a strip or plate reader.

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells. Samples containing less color than a calibrator will have a concentration of brevetoxin greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration of brevetoxin less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y axis) versus the log of the calibrator concentration (X axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the X axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.1 ppb or >2.5 ppb, respectively. Alternatively, Beacon can supply a spreadsheet template that can be used for data reduction. Please contact Beacon for further details.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD \pm SD*	%RSD	%Bo**	Brevetoxin conc. (ppb)
0.0 ppb Calibrator	1.71 1.72	1.72 \pm 0.010	0.58	100	0.00
0.1 ppb Calibrator	1.35 1.34	1.35 \pm 0.003	0.25	78	0.10
0.25 ppb Calibrator	0.98 0.99	0.99 \pm 0.011	1.16	58	0.26
0.5 ppb Calibrator	0.70 0.73	0.72 \pm 0.016	2.29	42	0.51
1.0 ppb Calibrator	0.51 0.52	0.51 \pm 0.007	1.42	30	0.94
2.5 ppb Calibrator	0.31 0.31	0.31 \pm 0.001	0.42	18	2.69

Actual values may vary; this data is for example purposes only.

* Standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

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

GENERAL LIMITED WARRANTY

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