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Microcystin Tube Kit

Cat. # 520012

Instructional Booklet
Read Completely Before Use.

Intended Use

The Abraxis Microcystin Coated Tube Kit is an immunological laboratory test for the quantitation of Microcystins in water.

Test Principles

The Abraxis Microcystin Coated Tube Kit uses a polyclonal antibody that binds both Microcystins and a Microcystin-enzyme conjugate. Microcystins in the sample compete with the Microcystin-enzyme conjugate for a limited number of antibody binding sites. Secondary antibodies (anti-rabbit), which bind anti-Microcystins, are immobilized to the inside of the test tube. In the assay procedure you will:

- Add anti microcystin solution and a sample containing Microcystins to the coated test tube well, followed by Microcystin-enzyme conjugate. The conjugate competes with any Microcystins in the sample for the same antibody binding sites.
- Wash away any unbound molecules, after you incubate this mixture for 20 minutes.
- Add clear substrate solution to each well. In the presence of bound Microcystin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every tube, and each tube receives the same number of Microcystin-enzyme conjugate molecules, a sample containing a low concentration of Microcystins allows the antibody to bind many Microcystin-enzyme conjugate molecules. The result is a dark blue solution.

Conversely, a high concentration of Microcystins allows fewer Microcystin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to Microcystin concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

Sample Calculations

Well Contents	OD	Average OD ± SD**	%RSD	%Bo
Negative Control	1.067 1.072	1.070 ± 0.004	0.33	100
0.15 ppb Calibrator	0.896 0.905	0.901 ± 0.006	0.71	84.2
0.4 ppb Calibrator	0.738 0.749	0.744 ± 0.008	1.05	69.5
1.0 ppb Calibrator	0.578 0.563	0.571 ± 0.011	1.86	53.4
2.0 ppb Calibrator	0.399 0.396	0.398 ± 0.002	0.53	37.5
5.0 ppb Calibrator	0.271 0.272	0.272 ± 0.001	0.26	25.6

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

** Standard deviation

Technical Assistance

For questions regarding this kit or for additional information about Abraxis products, call (215) 357-3911.

Safety

To receive complete safety information on this product, contact Abraxis LLC and request Material Safety Data Sheets.

Calculate Results

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

   %B° = (average OD of calibrator, control or sample x 100) ÷ average OD of negative control

2. Graph the %Bo of each calibrator on the Y (linear) axis against its Microcystin concentration on the X (log) axis using semi-log graph paper. Draw the best-fit line through the calibrator points.

3. Determine the Microcystin concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.

2. Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

Quality Control

1. The value of the 0.75 ppb control should fall within the following range:

   0.75 ppb Microcystin control 0.56 – 0.94 ppb

Performance Characteristics

Specificity

The Abraxis Coated Tube Kit does not differentiate between Microcystin-LR (used as kit calibrators) and other microcystin variants, but detects their presence at varying degrees. The following table shows the relative values for 50% B_o and the percent cross-reactivity (%CR) versus Microcystin-LR. All concentrations are in parts per billion (ppb).

Variant		%CR
Microcystin-LR		100
Microcystin-RR		87
Microcystin-YR		48
Microcystins-LA		3
Nodularin		126

Precautions

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not freeze tube kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents and samples to reach ambient temperature before you begin the test.
- Do not use kit components after the expiration date.
- Do not mix reagents or test tubes from kits with different lot numbers.
- Use approved methodologies to confirm any positive results.

Materials Provided in the Abraxis Microcystin Tube Kit

This tube kit contains the following items:

40 antibody coated tubes

1 vial of Negative Control (0.0 ppb Microcystin-LR)

1 vial each of 0.15 ppb, 0.40, 1.0, 2.0 and 5.0 ppb Microcystin-LR Calibrator

1 vial 0.75 ppb microcystin control.

1 vial of Microcystin-HRP Enzyme Conjugate

1 vial of Microcystin antibody solution

1 vial of Substrate

1 vial of Stop Solution

1 vial of 100X Wash Solution

You also need these items:

- Photometer capable of reading optical density of 12 mm tubes at 450nm.
- Tape or Parafilm®
- Watch or timer
- Clean running water or a wash bottle containing deionized water.

Assay Procedure

1. Bring all kit reagents and samples to be run to room temperature.

2. Prepare 1X wash solution by diluting the 100X wash concentrate with DI water. 1 mL concentrate per 100 mL DI water.

3. Remove the required number of antibody coated tubes from the re-sealable foil bag. Place tubes in rack and label with samples or calibrator level. Be sure to re-seal the bag with the desiccant to limit exposure of the tubes to moisture.

4. Add **500 µL of Enzyme Conjugate** to each tube.

5. Pipet **500 µL of calibrators, control and samples** into the appropriate tubes. Be sure to use a clean pipet tip for each solution to avoid cross contamination.

6. Add **500 µL of Antibody Solution** to each tube.

7. Swirl the tubes rapidly to mix the contents.

8. Incubate for **20 minutes**.

9. After incubation, vigorously shake the contents of the tubes into a sink. Flood the tubes completely with wash solution, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the rack on absorbent paper and tap out as much water as possible.

10. Add **500 µL of Substrate** to each tube.

11. Cover the tubes and incubate for **20 minutes.**

12. Add **500 µL of Stop Solution** to each tube in the same order of addition as the Substrate.

WARNING: Stop Solution is 1N hydrochloric acid. Handle carefully.

13. Read the tubes with a spectrometer or tube reader at 450nm. If the reader has dual wavelength capability, read at 450nm minus 605 or 650nm.