

Importance of β -N-methylamino-L-alanine (BMAA) Determination

β -N-methylamino-L-alanine (BMAA) is a non-protein amino acid produced by several types of cyanobacteria found in freshwaters, marine waters, and soil. When ingested, BMAA damages and ultimately destroys motor neurons in the spinal cord, causing the same type of damage seen in patients with ALS, and causes neurofibrillary tangles in the spinal cord and brain, similar to those seen in Alzheimer's disease. Those suffering from this BMAA-induced damage are classified as suffering from amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC). The symptoms of ALS/PDC include the varying degrees of the muscular paralysis of ALS, the muscular rigidity of Parkinson's, and/or the dementia of Alzheimer's and ultimately result in death.

Humans may be exposed to BMAA through the ingestion of contaminated drinking water or foods. Drinking water may become contaminated with BMAA through the proliferation of BMAA-producing cyanobacteria in drinking water sources such as lakes and reservoirs. It is unknown whether the various methods of water treatment are able to remove BMAA from the drinking water supply. Exposure also occurs through ingestion of foods such as cycad seeds, on whose plant roots BMAA-producing cyanobacteria are known to live and thereby contaminate the plant and its seeds, and through the ingestion of fish or other animals which have consumed toxin containing plants or cyanobacteria. An example of this biomagnification was seen in Guam in the 1950s, when fruit bats, which consume cycad seeds, were consumed by the native Chamorro people causing a dramatic increase in cases of ALS/PDC, known on Guam as "lytico-bodig."

The ABRAXIS® BMAA ELISA allows for the analysis of 42 samples in duplicate determination. Less than 1 mL of sample is required. The test can be performed in approximately 2 hours.

Performance Data

Test sensitivity: The limit of quantitation for BMAA (95% B/B₀) is approximately 4 ng/mL. The middle of the test (50% B/B₀) is approximately 100 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.

Sample concentration may be performed for samples requiring a lower limit of detection (technical bulletin available from Gold Standard Diagnostics by request).

Sample extraction and clean-up are necessary for biological samples (please refer to Z. Spáčil, J. Eriksson, S. Jonasson, U. Rasmussen, L. L. Ilag and B. Bergman, *Analyst*, 2010, **135**, 127-132).

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Specificity: Cross-reactivity of the ABRAXIS® BMAA ELISA for related compounds:

β -N-methylamino-L-alanine (BMAA)	100%
L-Cysteine hydrochloride	0.2%
L-Glutamic acid	0.2%
L-Aspartic acid	0.2%
γ -Aminobutyric acid	0.02%
DL-2,4-Diaminobutyric acid dihydrochloride	0.01%

No cross-reactivity was seen with Glycine, L-Isoleucine, L-Lysine monohydrochloride, L-Histidine monohydrochloride monohydrate, L-Tryptophan, L-Alanine, L-Tyrosine, L-Valine, L-Cystine, L-Asparagine, L-Phenylalanine, L-Threonine, L-Proline, L-Arginine monohydrochloride, L-Glutamine, L-Methionine, trans-4-hydroxy-L-proline, L-Serine, and L-Leucine up to 1 μ g/mL.

No cross-reactivity was seen with Microcystin-LR and Cylindrospermopsin up to 0.1 μ g/mL.

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ABRAXIS® β -N-methylamino-L-alanine (BMAA) ELISA* Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of β -N-methylamino-L-alanine (BMAA) in Water Samples *US Patent 8,394,596
Product No. 520040

1. General Description

The ABRAXIS® β -N-methylamino-L-alanine (BMAA) ELISA is an immunoassay for the quantitative and/or qualitative screening of BMAA in water samples. **Note:** This assay is intended for Research Use Only.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of BMAA. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

3. Storage and Stability

The ABRAXIS® BMAA ELISA should be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box. The standards are supplied in lyophilized form and must be reconstituted before use (See Test Preparation, Section D). Reconstituted standards may be used for up to one month (store frozen).

4. Test Principle

The test is a direct competitive ELISA based on the recognition of BMAA by specific antibodies. BMAA, when present in a sample, and a BMAA-HRP analogue compete for the binding sites of the rabbit anti-BMAA antibodies in solution. The BMAA antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of BMAA present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the ABRAXIS® BMAA ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

The presence of the following substances were found to have no significant effect on the BMAA Kit: manganese sulfate and aluminum oxide up to 10,000 ppm; calcium chloride and sodium thiosulfate up to 1000 ppm; potassium phosphate, ferrous sulfate, and zinc sulfate up to 100 ppm; copper chloride, calcium sulfate, magnesium sulfate, sodium fluoride, and sodium nitrate up to 10 ppm; sodium chloride up to 1 ppm; humic acid and magnesium chloride up to 0.1 ppm; methanol up to 1%; and seawater up to 10%.

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The ABRAXIS® BMAA ELISA kit provides screening results. As with any analytical technique (GC/MS, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.

6. Working Instructions

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with a secondary antibody, in a resealable aluminum pouch
2. Lyophilized BMAA Standards (6): 0, 5, 25, 100, 250, 500 ng/mL (ppb), must be reconstituted before use, see Test Preparation (Section D)
3. Antibody Solution (rabbit anti-BMAA), 6 mL
4. BMAA-HRP Conjugate Solution, 6 mL
5. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
6. Sample Diluent, 25 mL
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-200 μ L)
2. Multi-channel pipette (50-250 μ L) or stepper pipette with disposable plastic tips (50-250 μ L)
3. Microtiter plate reader (wave length 450 nm)
4. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section D)
5. Deionized or distilled water
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or parafilm

C. Sample Collection and Storage

Water samples should be collected in clear glass, polyethylene terephthalate glycol (PETG), high density polyethylene (HDPE), Polycarbonate (PC), or Polystyrene (PS) containers. **Do not use amber glass containers, as analyte will be lost due to adsorption.**

Samples must be analyzed immediately or stored frozen to avoid degradation of toxin which could produce inaccurate results.

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The conjugate, antibody, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. The standards are provided in lyophilized form. To reconstitute, add 1 mL of deionized or distilled water to each standard vial. Vortex thoroughly. Reconstituted standards may be used for up to one month (**store frozen**).
5. Dilute the ABRAXIS® Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
6. The stop solution must be handled with care as it contains diluted H₂SO₄.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
(0; 5; 25; 100; 250; 500 ppb)

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	etc.									
B	Std 0	Std 4	etc.									
C	Std 1	Std 5										
D	Std 1	Std 5										
E	Std 2	Samp1										
F	Std 2	Samp1										
G	Std 3	Samp2										
H	Std 3	Samp2										

F. Assay Procedure

1. **Add 100 μ L of the reconstituted standards or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. **Add 50 μ L of enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. **Add 50 μ L of antibody solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
4. **Incubate the strips for 90 minutes at room temperature.**
5. After incubation, remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips four times using the diluted wash buffer.** Use at least a volume of 250 μ L of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. **Add 150 μ L of substrate (color) solution** to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. **Incubate the strips for 30 minutes at room temperature.** Protect the strips from direct sunlight.
7. **Add 100 μ L of stop solution** to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. Evaluation

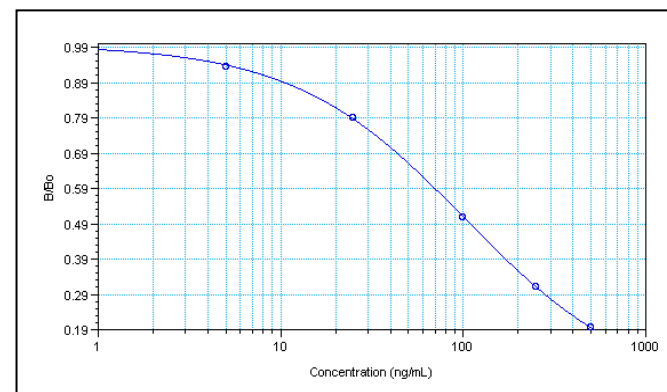
The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B₀ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B₀ for each standard on the vertical linear (y) axis versus the corresponding BMAA concentration on the horizontal logarithmic (x) axis on graph paper. %B/B₀ for samples will then yield levels in ppb (or ng/mL) of BMAA by interpolation using the standard curve. Results can also be obtained by using a spreadsheet macro available from Gold Standard Diagnostics upon request.

The concentrations of the samples are determined using the standard curve run with each test. Sample extracts showing a lower concentration of BMAA than standard 1 (5 ppb) should be reported as containing < 5 ppb of BMAA. Samples showing a higher concentration than standard 5 (500 ppb) must be diluted further with the provided sample diluent and re-analyzed.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards. Samples with lower absorbances than a standard will have concentrations of BMAA greater than the concentration of that standard. Samples which have higher absorbances than a standard will have concentrations of BMAA less than that standard.

As with any analytical technique ((GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

H. Standard Curve



For demonstration purposes only. Not for use in sample interpretation.