Importance of Carbamazepine Determination

One of the most frequently reported pharmaceuticals in surface water is carbamazepine. This drug is used as an anticonvulsant primarily in the treatment of epilepsy to control seizures. Other indications are attention-deficit disorder, bipolar disorder, and schizophrenia. Like most other pharmaceuticals, carbamazepine and its metabolites enter the water cycle through human excretions. In addition, a considerable amount of unused medication is flushed down the toilet and contributes to wastewater concentrations of pharmaceuticals.

Most of the household water in the civilized world is treated before it is allowed to enter the environment, but mishaps can occur and untreated water can escape into streams, etc. It is not always easy to identify when failure of WWTPs has occurred or when surface waters are contaminated from septic systems, especially if it was an accident or occurred in a remote or unmanned location. One way around this is to analyze the surface waters regularly to detect changes in the amounts of marker compounds.

Carbamazepine has been found in the effluent of wastewater treatment plants (WWTP) in concentrations up to 3.8 ug/L. Plants that use activated sludge, remove very little amounts of carbamazepine. Several studies have even reported an increase in carbamazepine concentration during treatment processes due to the cleavage of metabolic conjugates. Hence, carbamazepine is introduced into rivers and streams by WWTP discharge.

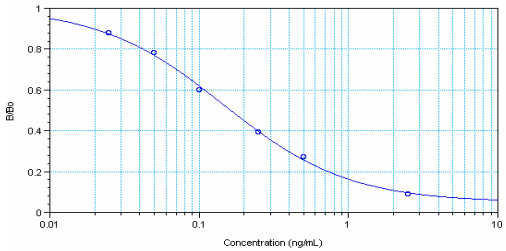
After entering the environment, carbamazepine has been shown to degrade very slowly by sunlight with a half-life of 100 days. It shows a moderate to low adsorption to soil. Being so persistent and almost ubiquitously present in environmental waters, carbamazepine has been proposed as a market for anthropogenic input to the aquatic environment.

Performance Data

Test sensitivity: The detection limit for this assay is 0.021 ppb (ug/L)

Test reproducibility: The standard curve R^2 must be ≥ 0.98. The Coefficient of variation (CVs) for standards should be <13% and for the CVs for samples should be <20%. Standard 0 absorbance value should be between 0.8 - 3.000.

Sensitivity: The assay exhibits very good cross-reactivity with Carbamazepine and not with other non-related compounds tested.



Cross-reactivities:		
	Carbamazepine (CBZ)	100% (per definition)
	10, 11-Dihydro CBZ	97%
	10, 11-Epoxy CBZ	78%
	2, Hydroxy CBZ	13%
	Protriptylene	8.6%
	Amitryptylene	4.1%
	Opipramol	3.9%
	Imipramine	3.6%

The following compounds had cross-reactivity of < 1%: Doxepine; ox CBZ; 10,11-Dihydro-10-Hydroxy CBZ; Iminostilbene; 10,11-Dihydro-trans, 10,11-dihydro CBZ; Acridine.

References

(1) Yu L., Fink G., Wintgens T., Melin T., Ternes TA. (2005). *Water Res.* 43, 951-960.

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ABRAXIS® Carbamazepine ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Carbamazepine in Water Samples

Product No. 515585

1. General Description

The ABRAXIS® Carbamazepine ELISA is an immunoassay for the quantitative and sensitive detection of Carbamazepine in water samples. A pre-sample concentration is not required. If necessary, positive samples can be confirmed by HPLC, or other conventional methods.

2. Safety Instructions

The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Carbamazepine ELISA should be stored in the refrigerator (2-8°C). Solutions should 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 expiration date on the box. Consult state, local and federal regulations for proper disposal of all reagents.

4. Test Principle

The test is a direct competitive ELISA that allows the detection of Carbamazepine. It is based on the recognition of Carbamazepine by specific antibodies. Carbamazepine, when present in a sample, and a Carbamazepine-HRP analogue compete for the binding sites of mouse anti-Carbamazepine antibodies in solution. The Carbamazepine antibodies are then bound by a second antibody (goat anti-mouse) immobilized in the 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 the Carbamazepine 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® Carbamazepine ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects cannot be completely excluded. The presence of the following substances up to 10,000 ppm were found to have no significant effect on the Carbamazepine Assay results: aluminum oxide, calcium chloride, calcium sulfate, manganese sulfate, magnesium sulfate, magnesium chloride, sodium chloride, phosphate, sodium thiosulfate, sodium nitrate. Copper Chloride, zinc sulfate, ferric sulfate, sodium fluoride up to 1,000 ppm. Humic acid up to 10 ppm.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit, wrong 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 during the test performance (lower than 10°C or higher than 30°C). The assay procedure should be performed away from direct sun light.

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

6. Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (goat anti mouse).
2. Standards (7): 0, 0.025, 0.050, 0.10, 0.25, 0.50, 2.5 ng/mL.
3. Antibody solution (mouse anti-Carbamazepine), 6 mL
4. Carbamazepine-HRP, 6 mL
5. Diluent/zero, 25 mL. Use to dilute samples with concentration above 2.5 ppb.
6. ABRAXIS® Wash Buffer 5X Concentrate, 100 mL
7. Color Solution (TMB), 12 mL
8. Stop Solution, 6 mL

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

1. Micro-pipettes with disposable plastic tips (50-250 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Reagent reservoir for multichannel pipettes
4. Microtiter plate washer (optional)
5. Microtiter plate reader (wavelength 450 nm)
6. Shaker for microtiter plates (optional)

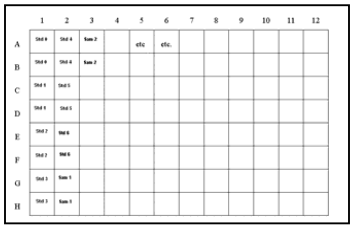
C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, enzyme conjugate, substrate solution, and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination. Read and understand the instructions and precautions given in this insert before proceeding.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard, control, antibody solution, enzyme conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The wash buffer is a 5X concentrated solution and needs to be diluted with deionized water. In a 1L container, dilute the 5X buffer 1:5 (i.e. 100 mL of the 5X wash solution plus 400 mL of deionized water). The diluted solution is used to wash the microtiter wells.
5. The stop solution has to be handled with care as it contains diluted H_2SO_4.

D. Working Scheme

The microtiter plate consists of 12 strips of 8, which can be used individually. 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 6: Standards
Sam1, Sam2, Sam3, etc.: Samples

E. Assay Procedure

1. **Add 75 µL of the standard solutions, control or samples** into the wells of the test strips according to theworking scheme given. We recommend using duplicates or triplicates.
2. **Add 50 µL of the enzyme conjugate solution** to the individual wells successively using a multi-channelpipette or a stepping pipette.
3. **Add 50 µL 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 holderin a rapid circular motion on the bench top for about 30 seconds. Be careful not to spill contents. **Incubate the strips for ninety (90) minutes at 2-8 °C.**
4. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. **Wash the strips four times using the 1X wash buffer solution.** Please use at least a volume of 250 µL of wash buffer for each well and each washing step. Remaining buffer in the wells should be removed bypatting the plate dry on a stack of paper towels.
5. **Add 100 µL of substrate/color solution** to the wells using a multi- channel pipette or a stepping pipette. **Incubated the strips for 30 minutes at room temperature.** Protect the strips from sunlight.
6. **Add 50 µL of stop solution** to the wells in the same sequence as for the substrate/color solution using a multi- channel pipette or a stepping pipette.
7. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after stopping the reaction.

F. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameters, Logit/Log or alternatively point to point). 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 a vertical linear (y) axis versus the corresponding Carbamazepine concentration on horizontal logarithmic (x) axis on graph paper. %B/B0 for controls and samples will then yield levels in ppb of Carbamazepine by interpolation using the standard curve. The concentrations of the samples are determined using the constructed standard curve (do not use a previously stored curve). Samples showing a lower concentration than 0.025 ppb of Carbamazepine should be reported as < 0.025 ppb. Samples showing a higher concentration than standard 6 (2.5 ppb) must be diluted to obtain more accurate results. The concentrations of the samples are determined using the constructed standard curve (do not use a previously stored curve). Samples showing a lower concentration than 0.025 ppb of Carbamazepine should be reported as < 0.025 ppb. Samples showing a higher concentration than standard 6 (2.5 ppb) must be diluted to obtain more accurate results.