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DOMOIC ACID ELISA TEST KIT (520505) VALIDATION

Domoic acid (ASP or amnesic shellfish poisoning) is a naturally occurring marine biotoxin associated with the "bloom" of diatoms primarily of the genus Pseudo-nitzschia but it is also produced by members of the genera Amphora and Nitzschia as well as some red algae. Crustaceans, fish and shellfish are capable of accumulating elevated levels of the toxin without adverse effects to themselves but high levels of the toxin does pose a significant risk to humans if contaminated fish tissue is consumed. The health impacts include vomiting, diarrhea, abdominal pain, disorientation, memory loss, seizures, and death. This test is suitable for the quantitative detection of Domoic Acid in water and seawater samples as well as shellfish samples. The US FDA, Health Canada, and EU have established a regulatory limit in shellfish of 20 µg/g.

Test kit validation provides an assurance of reliability during normal use and provides documented evidence that the method does what it is intended to do. Validations have been completed to ensure that Eurofins Abraxis' Domoic Acid (ASP) ELISA 96 well test kits are accurate, precise, specific, reproducible and robust. The performance results presented here demonstrate

- Limit of Detection (LOD)/Sensitivity
- Limit of Quantitation (LOQ)/Precision
- Lot-to-lot Consistency
- Specificity

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DOMOIC ACID TEST METHOD

Enzyme-Linked Immunosorbent Assay (ELISA) 96 well plate kit.

This method is a direct competitive ELISA based on the recognition of Domoic Acid by specific antibodies. The standards (prepared from certified reference materials), control and samples are added to and analyzed in the ELISA microtiter plate. This method allows for the detection and quantification of Domoic Acid between of 0.0065 to 10.0 ppb in fresh water. The assay can be run and results obtained in less than 2 hours, including sample preparation.

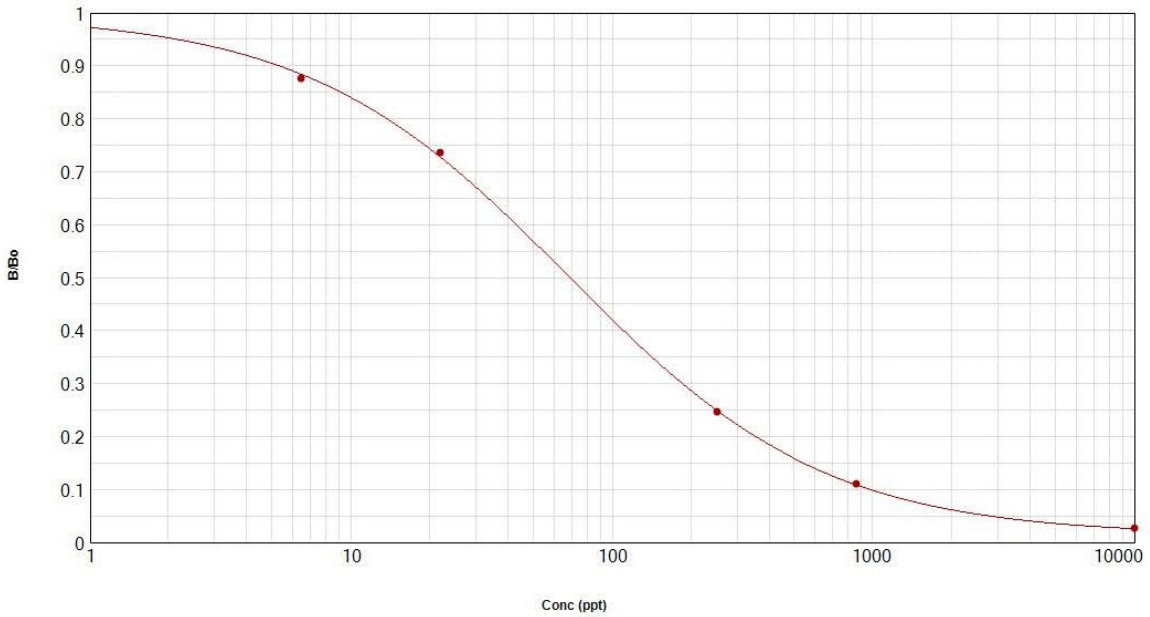
SAMPLE MATRICES

For the performance validation study, extracts from various sample matrices were evaluated for Domoic Acid using the Eurofins Abraxis Domoic Acid ELISA test kit method.

- Water (two fresh raw water samples)
- Seawater
- Clams

LIMIT OF DETECTION (LOD)/SENSITIVITY

The Domoic Acid ELISA has an estimated LOD (90% B/B0) of 0.0065 ppb (µg/L) in water (0.325 ppb in seawater and 13 ppb in shellfish compensating for matrix dilution). The middle of the calibration curve (50% B/B0) is approximately 0.075 ppb (3.75 ppb in seawater and 150 ppb in shellfish, compensating for matrix dilution) as demonstrated by the curve below. Determinations closer to the middle of the calibration curve give the most accurate results.





LIMIT OF QUANTITATION (LOQ)/PRECISION

Validated LOQ values were determined by spiking Domoic Acid gravimetrically into sample matrix (seawater, clams and two fresh raw water samples) to approximate these concentrations. Ten replicates of each sample were then analyzed by the ELISA method.

Results: Various sample matrices (seawater, clams and 2 fresh raw water samples) were spiked at 6.5 ppt (0.0065 ppb). % CV across each sample group was less than 10% and spike recoveries were +/- 25% of the spiked amount.

Sample	Spike (ppt)	Result	% Recovery
Seawater	6.5	6.72	103.3
	6.5	7.06	108.6
	6.5	7.31	112.4
	6.5	7.28	112.0
	6.5	6.77	104.2
	6.5	7.60	116.9
	6.5	7.64	117.5
	6.5	7.06	108.6
	6.5	6.88	105.9
	6.5	7.63	117.3
	CV:	4.88	

Sample	Spike (ppt)	Result	% Recovery
Fresh Raw Water Sample 1	6.5	5.76	88.6
	6.5	7.30	112.4
	6.5	6.47	99.5
	6.5	5.92	91.1
	6.5	5.84	89.8
	6.5	5.80	89.2
	6.5	7.08	108.9
	6.5	5.96	91.7
	6.5	5.80	89.2
	6.5	6.05	93.0
	CV:	9.11	

Sample	Spike (ppt)	Result	% Recovery
Clam	6.5	6.97	107.3
	6.5	6.55	100.7
	6.5	7.94	122.1
	6.5	7.15	110.0
	6.5	7.34	112.8
	6.5	6.77	104.1
	6.5	7.22	111.1
	6.5	7.75	119.2
	6.5	6.55	100.7
	6.5	7.45	114.7
	CV:	6.59	

Sample	Spike (ppt)	Result	% Recovery
Fresh Raw Water Sample 2	6.5	5.84	89.8
	6.5	6.34	97.5
	6.5	5.76	88.6
	6.5	7.30	112.4
	6.5	6.13	94.3
	6.5	6.05	93.0
	6.5	5.80	89.2
	6.5	5.92	91.1
	6.5	5.76	88.6
	6.5	6.47	99.5
	CV:	7.80	

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LOT-TO-LOT CONSISTENCY

Lot-to-lot variation is a frequent challenge that limits a user or laboratory's ability to produce consistent results over time. Assuring lot-to-lot consistency is important to a successful testing program. The Domoic Acid control for each kit lot was evaluated for product consistency through quantitation in three different ELISA test kit lots. All samples, standards and controls were analyzed in duplicate per kit instructions.

Results: The % CV for the control run in each kit lot are statistically consistent between all the kit lots, showing excellent reproducibility.

	Control	Result	% Recov.	Cv
Lot 21JAN2022	75 ppt	84.76	113.01	0.8%
Lot 10FEB2022	75 ppt	68.48	91.31	1.1%
Lot R22F0800	75 ppt	80.27	107.03	0.8%

SPECIFICITY

The cross reactivity to other common cyanotoxins/compounds tested at the concentrations below presented no false positives.

Saxitoxin	up to 10 ppb
PbTx-2	up to 10 ppb
Okadaic Acid	up to 10 ppb
Glutamine	up to 100 ppb
Glutamic Acid	up to 100 ppb
Proline	up to 100 ppb

MANUAL TEST PROCEDURE

Domoic Acid ELISA tests can be run manually with laboratory equipment that includes pipettes, a microplate reader and other materials indicated in the test kit user's guide.

Part Number	Product Description
520505	Domoic Acid (ASP) ELISA 96-test kit)

Eurofins Abraxis
Phone I (215) 357-3911
E-mail I info.ET.Warminster@eurofinsUS.com
Website I www.abraxiskits.com

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