Doc. 2007A

Domoic Acid Screening Test Kit

Colorimetric Immunoassay
for the detection of
Domoic Acid
in environmental samples

Instructions and User Guide

FOR SCIENTIFIC RESEARCH USE

Manufactured by
Mercury Science Inc.
Tel: (866) 861-5836

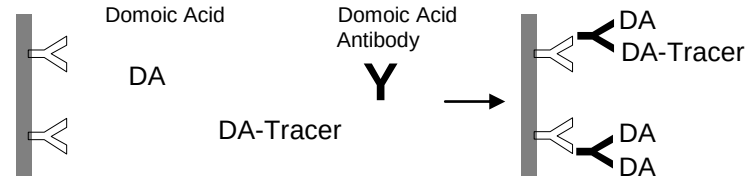
Domoic Acid Screening Test Kit

For Scientific Research Use Only.
This product is not to be used for In Vitro or In Vivo Diagnosis.

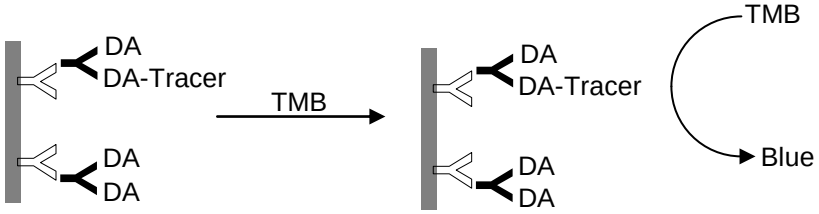
PRINCIPLES OF THE ASSAY
This product contains an antibody (Ab) that binds Domoic Acid and has been developed for the semi-quantitative detection of Domoic Acid in sample extracts. The signal of samples and a control are compared to determine the amount of Domoic Acid present.

The Domoic Acid assay is a solid phase colorimetric immunoassay, based on competition between Domoic Acid and enzyme-labelled Domoic Acid (DA-Tracer) for anti-Domoic Acid antibody. Samples containing Domoic Acid inhibit the binding of the DA-Tracer to the antibody molecules. Both the Ab-Domoic Acid and Ab-DA-Tracer complexes are captured on the surface of the microtiter plate wells.

Following a wash step, the addition of an enzyme substrate (TMB) forms a color proportional to the amount of DA-Tracer in the well. The amount of color measured is inversely proportional to the concentration of Domoic Acid in the sample.



Solid phase
anti-mouse IgG



TEST KIT CONTENTS Each Domoic Acid test kit contains reagents for testing a maximum of 36 samples in duplicate.
The expiry date of the test kit is stated on the outer label.
Store the kit between 2°C and 8°C.

Reagents Store the reagents between 2°C and 8°C when not in use.

Component	Quantity	
Control Solution	1 vial	2 mL
The control is a phosphate-buffered salt solution with casein. Contains sodium azide as a preservative.		
Sample Dilution Buffer	1 bottle	50 mL
Ready-to-use phosphate buffered (pH 7.8) salt solution with casein. Contains sodium azide as a preservative.		
Domoic Acid- Tracer	1 vial	7.5 mL
The tracer is in a MOPS-buffered solution containing bovine protein as a stabilizer and methylisothiazolone, bromonitrodioxane, and Proclin 300 as preservatives.		
Domoic Acid Antibody	1 vial	7.5 mL
The antibody is in phosphate-buffered salt solution with casein. Contains sodium azide as a preservative.		
Wash Concentrate	1 bottle	40 mL
A 25-fold concentration of Tris-HCI buffered (pH 7.8) salt solution with Tween 20. Contains sodium azide as a preservative. Prepare for use by mixing entire contents with 960 mL of distilled water and placing in platewasher WASH Bottle.		
Substrate Solution	1 bottle	15 mL
Tetramethylbenzidine and H_2O_2	Keep away from direct sunlight.	
Stop Solution	1 bottle	15 mL
1 N Hydrochloric Acid		
Anti-Mouse IgG Microtitration Strips	1 plate (12 x 8 wells)	

WARNINGS AND PRECAUTIONS

For research use only. Handle all samples as potentially hazardous.
Disposal of all waste should be in accordance with local regulations.

SCREENING ASSAY PROCEDURE

Perform each determination in duplicate for the Control and unknowns. All sample extracts should be filtered prior to analysis. All reagents and samples should be brought to room temperature prior to use. Use only the number of strips needed. Keep unused strips stored in their aluminum foil pouch with the included desiccant until needed.

1. Pipet 50 uL of the diluted Domoic Acid Antibody solution into each well.

2. Pipet 50 uL of each Control or sample into a well using the sequence shown in the table below. **Always use wells A and B on each strip as Controls.** Always perform duplicate analyses of samples. Three samples can be tested per strip. The example below shows the testing of eight samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Con-trol	Con-trol	Con-trol									
B	Con-trol	Con-trol	Con-trol									
C	1st Unk	4th Unk	7th Unk									
D	1st Unk	4th Unk	7th Unk									
E	2nd Unk	5th Unk	8th Unk									
F	2nd Unk	5th Unk	8th Unk									
G	3rd Unk	6th Unk										
H	3rd Unk	6th Unk										

3. Shake the wells for 30 minutes.

4. Pipet 50 uL of the Domoic Acid Tracer solution into each well.

5. Shake the wells for 30 minutes.

6. Wash the strips 3 times on the platewasher. Tap the strips upside-down firmly on a paper towel to blot away any excess wash solution that may remain in the wells.

7. Add 100 uL of Substrate Solution to each well. Shake the plate for five minutes.

8. Add 100 uL of Stop Solution to each well. Shake the plate briefly.

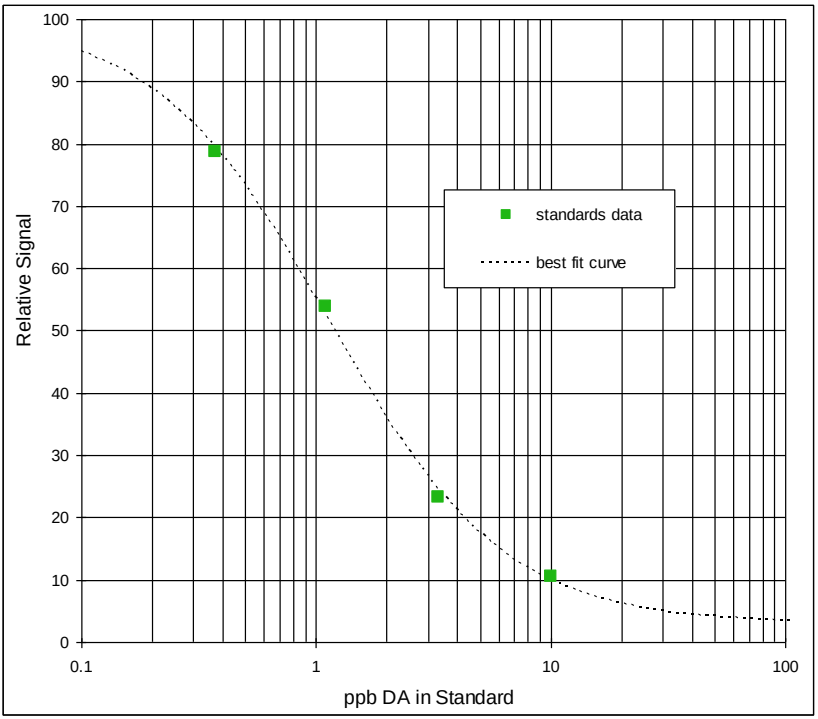
9. Measure the absorbance in each well. Note: If Control absorbance is greater than 3.0 AU, remove 50 uL from ALL WELLS and measure absorbance.

10. The data can be analyzed using the Excel worksheet available at the following link:

http://mercuryscience.com/Domoic Acid Quantitation 8Well Strip.xls

PERFORMANCE CHARACTERISTICS

Reproducibility
Inter-Assay Standard Curve
The average values and standard deviation of 5 separate standard curves is shown below.



Intra-assay Signal Precision
Analysis of 12 replicates for five different samples

	A	B	C	D	E
Signal (% of Control)	99.5	76.5	47.5	23.5	10.4
Standard Deviation	1.4	1.2	2.0	2.3	1.1
% Coeff. Var.	1.4	1.6	4.2	9.8	10.9

Intra-assay Concentration Precision
Analysis of 3 different samples measured in 6 separate quantitative assays.

	A	B	C
Average Conc. (ppb)	0.56	1.54	3.66
Standard Deviation (ppb)	0.01	0.13	0.19
% Coeff. Var.	2.1	8.6	5.3

PERFORMANCE CHARACTERISTICS (Cont.)

Detection Limit

The detection limit is defined as the minimum concentration of Domoic Acid that can be distinguished from a blank standard with 95% confidence. A detection limit of 0.1 ppb Domoic Acid in extraction buffer has been demonstrated with this assay.

Cross Reactivity

This assay is specific for the detection of domoic acid. The ability of the assay to detect structurally related compounds is shown in the following table.

Analyte	% Reactivity
Domoic Acid	100
Kainic Acid	0.3
Glutamic Acid	less than 0.1
Glutamine	less than 0.1

PROCEDURAL NOTES

Please read all instructions thoroughly before using this kit. Do not mix reagents from kits having different lot numbers. Do not use kits after the expiration date printed on the kit label.

Reagents should be at room temperature when used.

During washing steps, check that each well is completely filled during wash solution additions. After washing is complete, invert the wells and tap them gently against a paper towel to remove excess liquid.

The platewasher should be rinsed with distilled water at the end of each day of use to prevent clogging of the dispensing and aspirating ports. Prime the platewasher with wash solution before the first wash each day.

Care must be taken during each step to prevent contamination of reagents and equipment. Do not use the same pipet tip in two different reagents.

For Technical Assistance, contact Mercury Science Inc: (866) 861-5836.

Additional Information

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT
The Domoic Acid test kit is part of a complete system of immunodiagnostic reagents and instrumentation. The system requires the following equipment.

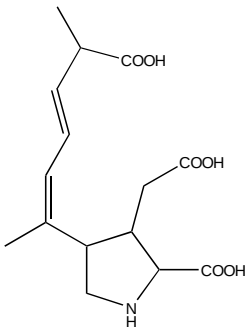
1. Microtiterplate Reader able to measure Absorbance at 450 nm
2. Platewasher
3. Plate Shaker
4. 8 Channel pipet
5. Pipetmen (P10, P200 and P1000)

Other Notes:

- Perform each Control and Sample in duplicate wells.
- All sample extracts should be filtered prior to analysis.
- All reagents and samples should be brought to room temperature prior to use.
- Use only the number of strips needed.
- Keep unused strips stored in their aluminum foil pouch with the included desiccant until needed.
- If Control absorbance is greater than 3.0 AU, remove 100 uL from ALL WELLS and repeat absorbance measurement.

An Excel worksheet has been developed to analyze results and quantitate the amount of domoic acid in extracts. Send your request for the "Domoic Acid Quantitation Worksheet - DAK-36" to: info@mercuryscience.com

Structure of Domoic Acid



Domoic Acid Test Kit

Summary Protocol Sheet

Add Antibody	**50 uL**
Add Control and Samples	**50 uL**
Incubate	**Shake for 30 minutes**
Add Tracer	**50 uL**
Incubate	**Shake for 30 minutes**
Wash	**"3 WASHES" program**
TMB	**Add 100 uL, shake for 5 minutes**
Stop	**Add 100uL**
Measure	**Absorbance at 450 nm**

Note: If Control absorbance is greater than 3.0 AU, remove 100 uL from ALL WELLS and repeat absorbance measurement.