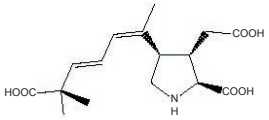
biosense LABORATORIES

Prod. no.A31300400-096

ASP Direct cELISA kit

for quantification of Domoic Acid



General

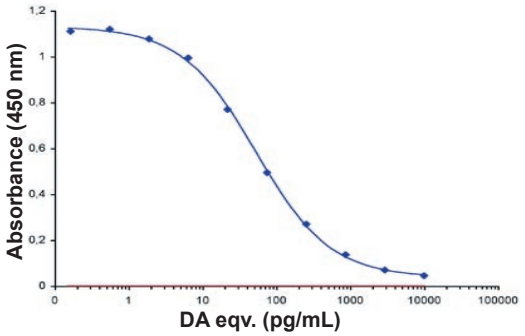
Domoic acid (DA) and DA isomers are water-soluble neurotoxins produced by a number of marine algae, in particular by the microalgae of the genus Pseudo-nitzschia. *Blooms of* Pseudo-nitzschia spp. *may lead to the accumulation of DA in shellfish filter feeders and other marine species [1,2]. Ingestion of DA contaminated shellfish may lead to amnesic shellfish poisoning (ASP) by affecting the central nervous system, and has caused the death of both animal and human consumers in severe cases [3]. The European Commission Directive 2002/226/EC implemented a maximum permitted level (MPL) of 20 mg DA/kg shellfish flesh intended for human consumption. This MPL is adopted by the regulatory authorities in most other countries.*

The Direct cELISA

Enzyme Linked Immunosorbent Assay (ELISA) has proved to be a sensitive and rapid method for detection of DA in the marine environment [4]. This quantitative DA ELISA was developed by AgResearch (Hamilton, New Zealand) for the detection of DA in water samples, shellfish and algal extracts, and is made from the antibodies developed by Garthwaite *et al.*,1998 [5]. The assay is primarily intended for use in routine monitoring of DA levels in cultured bivalve molluscs to comply with the regulatory MPL, but is also applicable for quantification of DA in other matrices.

The protocol

The kit protocol is based on a competitive binding assay where free DA and DA isomers in the sample are competing with a plate-coated DA-conjugated protein for binding to specific anti-DA antibodies. The anti-DA antibodies are labelled with horseradish peroxidase (HRP) and the amount bound in the wells is measured by addition of HRP substrate. The colour intensity is inversely proportional to the concentration of DA in the sample (Fig. 1). The assay is calibrated using a standard solution of DA supplied with the kit.



Assay performance

The assay is highly specific to DA, and is not interfered by structural analogues like kainic acid or L-glutamine derivatives. The assay has been subjected to an international collaborative validation study during spring 2003, and the results are being prepared for publication.

Assay analysis time	Working range (I_{80}-I_{20})	Assay LOQ	Shellfish sample LOQ
2,5 h	10-270 pg/mL	10 pg/mL	10 μg/kg shellfish

References

1) Bates, S.S. *et al.* (1998) In: *Physiological ecology of harmful algal blooms.* Springer Verlag, Heidelberg, *p.267-292.*
2) Scholin C.A. *et al.* (2002) *Nature 403, 80-84.*
3) Wright J.I.C. *et al.* (1989) *Can. J. Chem 67*, 481-490.
4) Garthwaite I., Ross K.M., Miles C.O., Briggs L., Towers N., Borell T. & Busby P. (2001) *J. AOAC 84, 1643-1648.*
5) Garthwaite I., Ross K.M., Miles C.O., Hansen R.P., Foster D., Wilkins A.L. & Towers N. (1998) *Nat. Toxins 6, 93-104.*

www.biosense.com
Biosense Laboratories AS, Thormøhlensgt. 55, N-5008 Bergen, Norway
Phone: +47 55543966, Fax: +47 55543771, e-mail: biosense@biosense.no