EuroProxima

VALIDATION REPORT

Tetrodotoxin Sensitive (TTXSENS) ELISA

Beijerinckweg 18
NL-6827 BN Arnhem
The Netherlands
Phone: +31 26 363 0 364
info@europroxima.com

Product code:
5191TTXSENS
Document no.:
TTXSENS[val01]02.22
Date: 22 February 2022

1. *Introduction*

Tetrodotoxin (TTX) and its analogues belong to a group of neurotoxins that are produced by various marine bacteria. The toxin can accumulate in certain species of fish, different marine bivalves (clams, oysters and mussels) and gastropods. Due to the worldwide increase in water temperature TTX has appeared also in the European waters. TTX has been recently detected in seafood harvested in the United Kingdom, Portugal, Spain, Greece and the Netherlands. The ingestion of contaminated seafood can have fatal consequences. On the cellular level TTX causes blockage of voltage-gated sodium channels that leads to alteration of neuronal functions and muscle paralysis. Death can occur due to heart or respiratory failure. The majority of the poisoning cases have been caused by the consumption of pufferfish contaminated with TTX in Japan, where a limit of 2000 µg/kg was set for fish. As for now there are no maximum limits for TTX in the European Union. According to the recent European Food Safety Authority Scientific Opinion the concentration of TTX and its analogues of 44 µg/kg of shellfish meat should not result in adverse effects in humans.

2. *Kit characteristics:* see manual 5191TTXSENS[1]02.22

The cross-reactivity pattern of the antibody:

Tetrodotoxin	100%
Okadaic acid	<0.1%
Saxitoxin	<0.1%
Domoic acid	<0.1%

3. *Scope of validation*

The validation study was carried out with the Tetrodotoxin Sensitive ELISA (5191TTXSENS). The study included:

- Determination of the limit of detection LOD in fish and shellfish samples
- Determination of the detection capability CCβ in fish and shellfish samples
- Determination of the % recovery in fish and shellfish samples
- Determination of inter- and intra- assay coefficients of variation

4. *Sample treatment*

Sample preparation was performed in accordance with the manual 5191TTXSENS[1]02.2022 Non-contaminated fish and shellfish (shrimp, prawn, mussels, crab, seafood mix) samples purchased locally were used.

5. Results

5.1 Limit of detection and detection capability

Table 1. Results of the validation study.

Sample	Procedure	TTXSENS ELISA kit	LOD (µg/kg)	CCβ (µg/kg)
Fish and shellfish	8.1	Batch 1	1.1	6
		Batch 2	1.7	
		Batch 3	1.4	
		Mean for 3 batches	1.4	6

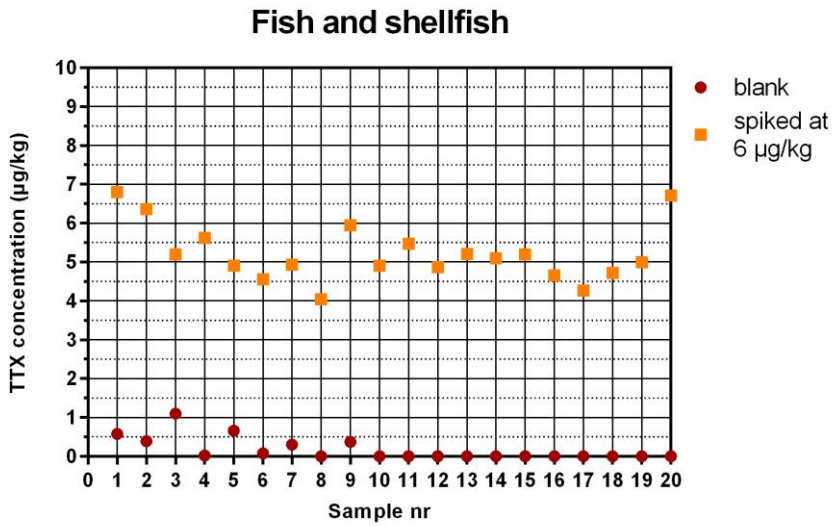

Fig. 1. Determination of CCβ of TTXSENS ELISA in fish and shellfish (ELISA kit batch 1).

5.2 Determination of recovery

Table 2. Recovery (%) determined for samples spiked at 6, 15 and 50 µg/kg with tetrodotoxin.

Sample	TTXSENS ELISA kit	Recovery (%)		
		6 µg/kg[1]	15 µg/kg[2]	50 µg/kg[2]
Fish and shellfish	Batch 1	87±12	101±7	92±9
	Batch 2	98±13	111±8	108±14
	Batch 3	97±17	102±8	102±14
	Mean for 3 batches	94±15	105±9	101±14

[1]n=20, [2]n=8

Product code: 5191TTXSENS
Document no.: TTXSENS[val01]02.22
Date: 22 February 2022

5.3 Determination of inter- and intra-assay coefficients of variation

Table 3. Intra- and inter-assay coefficients of variation for 0.625 ng/ml tetrodotoxin standard.

TTXS ELISA kit	Intra-assay[1] CV (%)	Inter-assay[2] CV (%)
Batch 1	2.2	4.4
Batch 2	2.6	3.7
Batch 3	3.2	3.9

[1]Mean of 48 results in duplicate

[2]Mean of 10 results in duplicate