

Mycotoxin Assay Handbook



Euroclone SpA - Life Science Division
Via Lombarida, 12 - 27010 Siziano (PV) ITALY
Customer service e-mail: info@euroclone.net
Technical Service e-mail: agalive@euroclone.net
Tel. +39-02-38195378; Fax +39-02-38195248

www.euroclone.net



ELISA Kits for the detection of mycotoxins in food

Local Distributor

Introduction

Cattle constitute one of the major livestock species and an important economical factor in many countries. The Mycotoxins are secondary metabolites of moulds, contaminating a wide range of crop plants and fruits before or after harvest, the most important mycotoxins being: Aflatoxins, Deoxynivalenol, Ochratoxin A, Fumonisin, Zearalenone, Patulin and T-2 Toxin.

The acute and chronic impact of mycotoxins on human and animal health is proven scientifically. Mycotoxin contamination is recognized as an unavoidable risk because the formation of fungal toxins is weather dependant and effective prevention is impossible.

According to the FAO more than 25 % of the world 's agricultural production is contaminated with mycotoxins. This equates to economic losses estimated at hundreds million annually in the grain industry alone. Most countries have adopted regulations to limit exposure to mycotoxins, having strong impact on food and animal crop trade. The presence of mycotoxins is unavoidable and therefore testing of raw materials and products is required to keep food and feed safe.

Recently, International Organizations such as CODEX Alimentarius, Food and Agriculture Organization (FAO) and World Health Organization (WHO) have joined in the difficult task of analyzing and establishing the criteria for mycotoxin risk assessment and harmonization of the related procedure to be applied worldwide.

In 2003, 7% of alert notifications according to the identified risk made by EU Notification Bodies were related to mycotoxin contamination, mainly due to aflatoxin (95%) in hazelnuts, peanuts, pistachio nuts and maize meal, Ochratoxin A (2%) in cocoa powder and Fumonisin (3%) in cereals.

Euroclone ELISA kits

Euroclone ELISA kits for the detection of mycotoxins in food are a reliable, rapid and sensitive assays. These products are very easy to use and they are recognized as a screening method for food control and monitoring issued by EU regulation.

Code	Description	Format	Samples
E11048	Total Aflatoxin, quantitative ELISA	1x96 tests	grain, nuts, cottonseed, animal feed
E11448	Ochratoxin A, quantitative ELISA	1x96 tests	grain, nuts, cottonseed, animal feed, coffee and wine
E11448D	Ochratoxin A WINE , semi-quantitative ELISA	1x48 tests	wine, must, fortified wine (cherry, porto)
E13148	Deoxyvalenol (DON), quantitative ELISA	1x96 tests	grain, cereals, cottonseed, animal feed
E95548	Aflatoxin M1, quantitative ELISA	1x96 tests	milk, milk powder, cheese



Aflatoxin M1 ELISA kit

Animals are exposed to aflatoxins by consumption of feeds that are contaminated by aflatoxin-producing fungal strains during growth, harvest or storage. When cows are fed contaminated feed, aflatoxin B1 is converted by hydroxylation to aflatoxin M1, which is subsequently secreted in the milk of lactating cows. Aflatoxin M1 is quite stable towards the normal milk processing methods such as pasteurization and if present in raw milk, it may persist into final products for human consumption. Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Many countries have declared limits for the presence of aflatoxin M1 in milk and milk products. In the EU the limit for the presence of M1 in milk and reconstituted milk powders has been set at 0.05 mg/L or 50 parts per trillion (50 ppt.)

Code	Description	Format	Samples
E95548	Aflatoxin M1, quantitative ELISA	1x96 tests	milk, milk powder, cheese

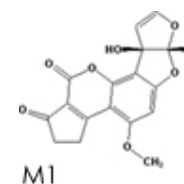
Kit content:

- **Microplate:** 96 wells microplate (12 eight well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody
- **Washing Buffer:** one pouch with PBS 0.05% Tween20®
- **Aflatoxin M1 standards:** 0,0/5,0/10/25/50/100 pg/ml (ppt) (6 vials of 3ml)
- **Aflatoxin M1 conjugate:** aflatoxin M1 conjugated to HRP (15ml) - ready to use
- **Substrate solution:** TMB (15ml) - ready to use
- **Stop solution:** Acidic Solution (15 ml) - ready to use

Kit features:

- **Samples:** milk, milk powder, cheese
- **Assay Principle:** Competitive and quantitative direct ELISA kit
- **Time Assay:** 2 hours and 20 minutes (alternative method: overnight incubation)
- **Cross Reactivity:** B1 100%, M1 79%, B2 76%, G1 55%, M2 33%, G2 6%***
- **Limit of detection:** 5ppt (for milk) and 25 ppt (for cheese)
- **EU Limits:** 10ppt (baby milk), 50 ppt (human)

*** Aflatoxin M1 is the predominant aflatoxin contaminant in cows milk so the kit is specific for the measurement of aflatoxin M1 in this matrix.



Chemical structure of Aflatoxin M1

- **High Sensitivity**
- **Quantitative Detection**
- **In line with EU limits**
- **No extraction for milk**

■ Deoxyvalenol (DON) ELISA kit

Deoxynivalenol (DON or vomitoxin) is a mycotoxin produced by certain species of *Fusarium*, the most important of which is *F. graminearum* (*Gibberella zeae*). This fungus causes Gibberella ear (also known as red ear rot) or stalk rot on corn and head scab in wheat. The fungus itself appears reddish to pinkish. The fungus may cause a reddish discoloration of the cob and kernels.

The mycotoxin deoxynivalenol causes reduced weight gain and suppresses animal feeding, especially in swine. At high concentrations (greater than 10 ppm) vomiting and total feed refusal may occur. FDA has recommended that total feed levels of DON not exceed 5 ppm for cattle and chicken, and 1 ppm for swine. DON levels for human food should be less than 1 ppm.

Red ear rot caused by *F. graminearum* is favored by warm wet weather after silking. Disease tends to be worse when corn is grown without rotation or after wheat as this pathogen also infects wheat. It may be worse when corn is grown in reduced tillage situations.

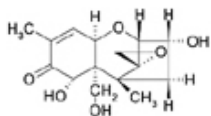
Code	Description	Format	Samples
E13148	Deoxyvalenol (DON), quantitative ELISA	1x96 tests	grain, cereals, cottonseed, animal feed

Kit content:

- **Microplate:** 96 wells microplate (12 eight well strips) in a microwell holder coated with a mouse anti-DON antibody
- **Microplate:** Dilution wells (green) 96 non-coated wells (12 eight well strips) in a microwell holder
- **DON standards:** 0,0/10/20/50/100/200 ng/ml (ppb) (6 vials of 1,5ml)
- **DON conjugate:** DON conjugated to HRP (2x12ml) - ready to use
- **Substrate solution:** TMB (15ml) - ready to use
- **Stop solution:** Acidic Solution (15 ml) - ready to use

Kit features:

- **Samples:** cereals, grains, cottonseed, animal feed, nuts
- **Assay Principle:** Competitive and quantitative direct ELISA kit
- **Time Assay:** 20 minutes (10 minutes for extraction protocol)
- **Cross Reactivity:** DON 100%
- **Limit of detection in samples:** 500 ppb
- **EU Limits:** 500-1750ppb (it depends on samples)



Chemical structure of Deoxyvalenol

Deoxynivalenol

- **Rapid Test**
- **Quantitative Detection**
- **In line with EU limits**

■ Total Aflatoxin ELISA kit

Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus* and *Aspergillus parasiticus*. They are carcinogenic and can be present in grains, nuts, cottonseeds and other commodities associated with human food or animal feeds. Crops may be contaminated by one or more of the four following sub-types of aflatoxin: B₁, B₂, G₁ and G₂. Aflatoxin B₁ is the most toxic and frequently detected form. The other types present a significant danger if the concentration is at a high level. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Rey's syndrome and chronic hepatitis. Animals are exposed to aflatoxins by consumption of feeds that are contaminated by aflatoxin producing fungal strains during growth, harvest or storage. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interference, immune suppression, decreased milk and egg production. Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Accurate and rapid determination of the presence of aflatoxin in commodities is of paramount importance.

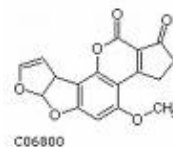
Code	Description	Format	Samples
E11048	Total Aflatoxin, quantitative ELISA	1x96 tests	grain, nuts, cottonseed, animal feed

Kit content:

- **Microplate:** 96 wells microplate (12 eight well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody
- **Microplate:** Dilution wells (green) 96 non-coated wells (12 eight well strips) in a microwell holder
- **Aflatoxin standards:** 0,0/0,2/0,5/1,0/2,0/4,0 ng/ml (ppb) (6 vials of 1,5ml)
- **Aflatoxin conjugate:** aflatoxin B₁ conjugated to HRP (2x12ml) - ready to use
- **Substrate solution:** TMB (15ml) - ready to use
- **Stop solution:** Acidic Solution (15 ml) - ready to use

Kit features:

- **Samples:** cereals, grains, cottonseed, animal feed, nuts
- **Assay Principle:** Competitive and quantitative direct ELISA kit
- **Time Assay:** 20 minutes (10 minutes for extraction protocol)
- **Cross Reactivity:** B₁-100%, B₂-77%, G₁-64%, G₂-25%
- **Limit of detection in samples:** 1ppb
- **EU Limits:**



Chemical structure of Aflatoxin B1

- **Rapid Test**
- **High Crossreactivity**
- **In line with EU limits**

■ Ochratoxin A ELISA kit

Ochratoxin A is a toxic metabolite produced by several molds of the *Aspergillus flavus* and *Penicillium* genera, including *Aspergillus ochraceus*. The fungal species has the potential to produce ochratoxin A, a known nephrotoxin and carcinogen. It has been frequently detected in human foods and animal feed, mainly in cereal products, although a range of commodities has been reported to contain the toxin. In humans, exposure to ochratoxin A has been linked with Balken endemic nephropathy (BEN), a chronic kidney disease associated with tumors of the renal system. In animals, impairment of renal function has been reported in swine. In turkeys and chickens symptoms included retarded growth, decreased feed conversion, nephropathy and mortality. Feed refusal has also been observed in turkeys. A decrease in egg production and shell quality was reported in both turkeys and chickens.

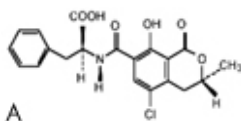
Code	Description	Format	Samples
E11448	Ochratoxin A, quantitative ELISA	1x96 tests	grain, nuts, cottonseed, animal feed, coffee and wine

Kit content:

- **Microplate:** 96 wells microplate (12 eight well strips) in a microwell holder coated with a mouse anti-Ochratoxin antibody
- **Microplate:** Dilution wells (green) 96 non-coated wells (12 eight well strips) in a microwell holder
- **Ochratoxin A standards:** 0,0/0,4/1,0/2,0/4,0/8,0 ng/ml (ppb) (6 vials of 1,5ml)
- **Ochratoxin A conjugate:** OTA conjugated to HRP (2x12ml) - ready to use
- **Substrate solution:** TMB (15ml) - ready to use
- **Stop solution:** Acidic Solution (15 ml) - ready to use

Kit features:

- **Samples:** cereals, grains, cottonseed, animal feed, nuts, coffee and wine
- **Assay Principle:** Competitive and quantitative direct ELISA kit
- **Time Assay:** 20 minutes (10 minutes for extraction protocol)
- **Cross Reactivity:** Ochratoxin A 100%, Ochratoxin B 9,3%
- **Limit of detection in samples:** 2 ppb
- **EU Limits:** 2ppb in cereals
- **Rapid Test**
- **High specificity**
- **In line with EU limits**
- **Suitable for a lot of matrices**



Chemical structure of Ochratoxin A

■ Ochratoxin A WINE ELISA kit

Ochratoxin A has been frequently detected in human foods and animal feed with the main human bioburden deriving from cereals and grain products, although a wide range of commodities has been found to contain the toxin. These include green and roasted coffee, cocoa, spices and grape derivatives such as raisins, grape juice and wines (Assessment of Dietary Intake of Ochratoxin A by the Population of EU Member States: Report of Experts Participating in Task 3.2.7, Jan 2002).

Code	Description	Format	Samples
E11448D	Ochratoxin A, semi-quantitative ELISA	1x48 tests	wine, must, fortified wine (cherry, porto)

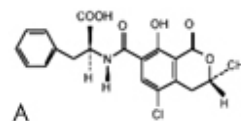
Kit content:

- **Microplate:** 48 wells microplate (6 eight well strips) in a microwell holder coated with a mouse anti-Ochratoxin antibody
- **Microplate:** Dilution wells (green) 48 non-coated wells (6 eight well strips) in a microwell holder
- **Ochratoxin A controls:** 0,0/1,0/2,0 ng/ml (ppb) (3 vials of 0,8ml)
- **Ochratoxin A conjugate:** OTA conjugated to HRP (2x12ml) - ready to use
- **Washing Buffer:** one pouch with PBS 0.05% Tween20@
- **Sample diluent:** proprietary buffer (12ml) - ready to use
- **Substrate solution:** TMB (6 ml) - ready to use
- **Stop solution:** Acidic Solution (6 ml) - ready to use

Kit features:

- **Samples:** red wine, white wine, must, sherry, porto, grape juice
- **Assay Principle:** Competitive direct ELISA kit
- **Time Assay:** 40 minutes
- **Cross Reactivity:** Ochratoxin A 100%, Ochratoxin B 9,3%
- **Limit of detection in samples:** 1-2 ppb
- **EU Limits:** 2ppb

- **No equipment**
- **No extraction**
- **Suitable for self control purposes**



Chemical structure of Ochratoxin A