Fumonisin B1 ELISA Kit (OKAO00145)

Lot# KC1512

Instruction for Use

For the quantitative detection of Fumonisin B1 (SDM) concentration in corn, feed and cooking oil.

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology Fumonisin B1 ELISA Kit (OKAO00145) is based on standard competitive inhibition enzyme-linked immuno-sorbent assay technology. Fumonisin B1 has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells along with a rabbit anti-Fumonisin B1 antibody and an anti-rabbit / HRP Conjugated antibody, then incubated. Wells are washed and detection substrates are added and incubated. An enzymatic reaction is produced through the addition of TMB which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm which is quantitatively proportional to the amount of detector antibody bound in the well and inversely proportional to the amount of Fumonisin B1 in the sample.

Background

Fumonisin B1 is the most prevalent member of a family of toxins, known as fumonisins, produced by several species of Fusarium molds, such as Fusarium verticillioides, which occur mainly in maize (corn), wheat and other cereals. Fumonisin B1 contamination of maize has been reported worldwide at mg/kg levels. Human exposure occurs at levels of micrograms to milligrams per day and is greatest in regions where maize products are the dietary staple. Fumonisin B1 is hepatotoxic and nephrotoxic in all animal species tested. The earliest histological change to appear in either the liver or kidney of fumonisin-treated animals is increased apoptosis followed by regenerative cell proliferation. While the acute toxicity of fumonisin is low, it is the known cause oftwo diseases which occur in domestic animals with rapid onset: equine leukoencephalomalacia and porcine pulmonary oedema syndrome. Both ofthese diseases involve disturbed sphingolipid metabolism and cardiovascular dysfunction.

General Specifications

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Range	0.5 – 40.5 ppb
LOD	< 0.5 ppb (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Fumonisin B1 CAS#: 116355-83-0 PubChem#: 3431 Alias: FB1; Fumonisin b1; Macrofusine; Fumonisin B1, Fusarium moniliforme; NSC629151; 116355-83-0; FB1
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Storage and Stability

• Upon receipt store kit at 4°C. Open kits may be stored for up to one month. Do not use past expiration date.

3. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Fumonisin B1 Microplate	96 Wells (12 x 8 strips)	Store at 4°C Open kits may be stored for up to one month Do not use past expiration date
Fumonisin B1 Standards	6 x 1 mL	
Fumonisin B1 Detector Antibody	1 x 6 mL	
HRP Conjugate	1 x 6 mL	
Substrate A	1 x 6 mL	
Substrate B	1 x 6 mL	
10X Sample Prep Buffer	2 x 20 mL	
20X Wash Buffer	1 x 20 mL	
Stop Solution	1 x 6 mL	

4. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.
• Heater water bath
• Centrifuge
• Reagents: Methanol, n-Hexane.

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

• To minimize influences on the assay performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

7. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

7.1 Fumonisin B1 Standards

Standard 0	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
0 ppb	0.5 ppb	1.5 ppb	4.5 ppb	13.5 ppb	40.5 ppb

7.2 70% Methanol

7.2.1 Immediately prior to use, prepare **70% Methanol** by diluting 100% Methanol 7:3 (V:V) using ultrapure water, with 7 parts Methanol and 3 parts water.

7.2.2 Mix gently and thoroughly.

7.2.3 Do not store for future use.

7.3 1X Sample Prep Buffer

7.3.1 Dilute 20 mL of the **2X Sample Prep Buffer** with distilled or deionized water at 1:1 to 40 mL (or the required volume) immediately prior to use.

7.3.2 Mix gently and thoroughly.

7.3.3 Do not store for future use.

7.4 1X Wash Buffer

7.4.1 If crystals have formed in the **20X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

7.4.2 Add the entire 20 mL contents of the **20X Wash Buffer** bottle to 380 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

7.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

7.4.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

7.5 Microplate Preparation

7.5.1 Micro-plates are provided ready to use and do not require rinsing or blocking.

7.5.2 Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

7.5.3 Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

8. Sample Preparation

8.1 Sample Preparation and Storage

- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Untreated samples should be stored at -20°C.
- Prepared samples can be stable for 1 week at 4°C.

8.1.1 Corn and Feed

8.1.1.1 Take 1g crushed samples and pipette 5 mL sample extract.

8.1.1.2 Mix for 5 min and Centrifugal at above 4000 r/min at room temperature (20-25°C) 10 min.

8.1.1.3 Transfer 0.1 mL supernatant (must be clear) into a new centrifuge tube, add 1.9 mL redissolving solution (1 x), mix properly for 2 min.

8.1.1.4 Take 50 µL for further analysis.

8.1.2 Cooking oil

8.1.2.1 Take 5 mL samples and pipette 5 mL sample extract and 8 mL n-hexane.

8.1.2.2 Mix for 5 min and Centrifugal at above 4000 r/min at room temperature (20-25°C) 10 min.

8.1.2.3 Remove supernatant, take 0.1 mL in the lower liquid, add 1.9 mL redissolving solution (1 x), mix properly for 2 min.

8.1.2.4 Take 50 µL for further analysis.

9. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Temperature regulation for all procedures and incubations to 25°C is recommended for optimal reproducibility.

9.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

9.2 Add 50 µL of standards or samples into wells of the **Fumonisin B1 Microplate**. At least two replicates of each standard, sample or blank is recommended.

9.3 Immediately add 50 µL of the **Fumonisin B1 Detector Antibody** and 50 µL of the **HRP Conjugate** to each well.

9.4 Cover the plate with the plate sealer, gently mix and incubate for 30 minutes at room temperature.

9.5 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

9.6 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.7 Wash plate 5 times with **1X Wash Buffer** as follows:

9.7.1 Add 250 µL of **1X Wash Buffer** to each assay well.

9.7.2 Incubate for 30 seconds.

9.7.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

9.7.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.7.5 Repeat steps 10.7.1 through 10.7.4 **four** more times.

9.8 Add 50 µL of prepared **Substrate A** and 50 µL of **Substrate B** to each well.

9.9 Gently mix the plate and incubate for 15 minutes **in the dark**.

9.10 Add 50 µL of **Stop Solution** to each well and mix gently. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for **Substrate A** and **Substrate B**.

9.11 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.10. If wavelength correction is available, set to 540 nm or 570 nm.

10. Calculation of Results

For analysis of the assay results, first derive the **Percent Relative Absorbance** for each sample or standard by calculating the mean absorbance between replicate wells. This value is then divided by the mean replicate Blank (0 ppb) absorbance, then multiplying by 100%:

$$\text{Percent Relative Absorbance (Sample or Standard)} = \frac{\text{Mean Absorbance (Sample or Standard)}}{\text{Mean Blank Absorbance}} \times 100\%$$

A standard curve is generated by plotting the **Percent Relative Absorbance** of each standard serial dilution point vs. the respective standard concentration. The Fumonisin B1 concentration contained in the samples can be interpolated by using linear regression of each sample **Percent Relative Absorbance** against the standard curve. This is best achieved using curve fitting software.

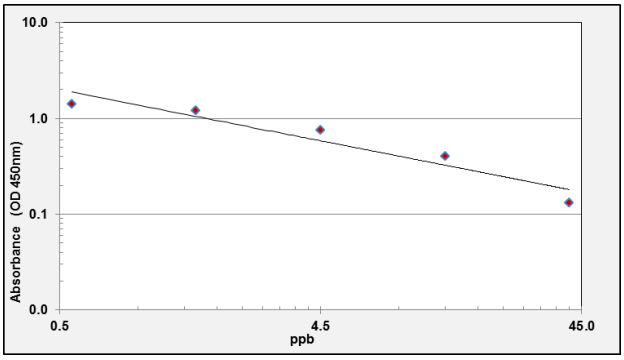
Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

11. Typical Expected Data

11.1 Typical Data

For convenience in result calculation, absorbance as abscissa and standard conditions can be used as ordinate. The standard curve in the manual is only for reference; experimenters should draw the standard curve based on their own data.



ppb	Absorbance		Mean Absorbance	Blank Subtracted
	Rep 1	Rep 2		
40.5	0.131	0.131	0.131	0.081
13.5	0.402	0.383	0.393	0.343
4.5	0.753	0.798	0.776	0.726
1.5	1.212	1.191	1.202	1.152
0.5	1.426	1.662	1.544	1.494
0.0	1.882	1.947	1.915	1.865

11.2 Limit of Detection

Derived by linear regression of OD450 of the Mean Blank + 2xSD

Sample	Mean Recovery
Corn, Feed	50 ppb
Cooking oil	10 ppb

11.3 Reproducibility

Three samples of known concentrations were measured in 20 replicates within an assay plate to assess intra-assay reproducibility. Three samples of known concentrations were measured across 40 replicate assays inter-assay reproducibility:

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ppb)	0.358	0.924	1.447	0.408	1.117	1.503
SD	0.024	0.066	0.113	0.029	0.09	0.134
CV (%)	6.7	7.1	7.8	7.1	8.1	8.9

11.4 Cross-Reactivity Rate

Substance	Cross Reactivity Rate
Fumonisin B1	100%

11.5 Recovery

The recovery of Fumonisin B1 spiked at levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample	Number	Mean Recovery
Corn	10	100 ±15%
Feed	10	95 ±15%
Cooking oil	10	85 ±15%

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
5754 Pacific Center Blvd, Suite 201
San Diego, CA 92121

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

Technical support: techsupport@avivasysbio.com

China

Beijing AVIVA Systems Biology
6th Floor, B Building, Kaichi Tower
#A-2 Jinfu Road.
Daxing Industrial Development Zone
Beijing, 102600, CHINA

Phone: (86)10-60214720
Fax: (86)10-60214722
E-mail: support@avivasysbio.com.cn

中国地址：北京大兴工业开发区金辅路甲 2 号凯驰大厦 B 座 6 层 (102600)
电话: 010-60214720/21
传真: 010-60214722

产品售前咨询及销售: sales@avivasysbio.com.cn
售后及技术支持: support@avivasysbio.com.cn