



## QUANTITATIVE ASSAY FOR – AFLATOXIN M<sub>1</sub> IN URINE

Cat.No. 991 AFLMO1U

### **AFLATOXINS**

Aflatoxins are toxic and carcinogenic secondary metabolites of the fungi *Aspergillus flavus* and *aspergillus parasiticus*. Chronic consumption of aflatoxins has been associated with primary liver cancer<sup>1</sup> and in sufficiently high doses, acute liver failure and death. While their presence in food commodities destined for animals or humans is strictly monitored, accidents and mistakes still occur, particularly in areas of the world with limited technological infrastructure<sup>2</sup>. Lethal outbreaks of aflatoxin poisoning (aflatoxicosis) have been reported recently in Africa<sup>3</sup> and in the West there has been an outbreak of aflatoxicosis in dogs, linked to contaminated dog food<sup>4</sup>. Aflatoxins have also been considered as a possible agent of bioterrorism<sup>5</sup>. After ingestion of aflatoxin B<sub>1</sub>, the most potent and prevalent of the aflatoxins, a portion is converted to the less toxic metabolite aflatoxin M<sub>1</sub> which is excreted in the urine. It has been shown that there is a good correlation between ingested aflatoxin B<sub>1</sub> and the appearance of aflatoxin M<sub>1</sub> in urine. The conversion rate is estimated to be about 2%<sup>6</sup>.

### **INTENDED USE**

The Helica Aflatoxin M<sub>1</sub> in urine assay is an enzyme-linked immunosorbent assay for the quantitative determination of aflatoxin in urine at levels which should be helpful in monitoring populations at risk for acute or chronic aflatoxicosis. The assay has not yet been approved by FDA for diagnostic purposes.

### **ASSAY PRINCIPLE**

The Helica Aflatoxin M<sub>1</sub> in urine assay is a direct enzyme-linked immunosorbent assay in which an antibody with high affinity for aflatoxin M<sub>1</sub> is coated onto polystyrene microwells. After an initial dilution with distilled water, the urine sample is mixed with assay buffer and added to the well. If aflatoxin M<sub>1</sub> is present in the urine it will bind to the coated antibody. Subsequently, aflatoxin bound to horse-radish peroxidase (HRP) is added and binds to the antibody not already occupied by aflatoxin present in the sample or standard. After this incubation period, the contents of the wells are decanted, washed and an HRP substrate is added which develops a blue color in the presence of enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of aflatoxin in the standard or the sample. Therefore, as the concentration of aflatoxin in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added which changes the chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm (OD<sub>450</sub>). The optical densities of the samples are compared to the OD's of the kit standards and a result is determined by interpolation from the standard curve.

### **Reagents Provided**

1 pouch: Antibody coated microwells	96 wells (12 eight well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody
1 plate: mixing wells (red)	96 non-coated wells (12 eight well strips) in a microwell holder
6 vials: Aflatoxin Standards	1.0ml/vial of aflatoxin M <sub>1</sub> at the following concentrations: 0.0, 0.15, 0.40, 0.80, 1.50, and 4.00 ng/mL in stabilized normal human urine
1 bottle: Aflatoxin HRP-conjugate	12 ml of aflatoxin conjugated to peroxidase in buffer with preservative
2 bottles: Assay Buffer	2 x 12ml propriety assay buffer
1 bottle: Substrate Solution	12ml stabilized tetramethylbenzidine (TMB)
1 bottle: Stop Solution	12ml Acidic Solution
1 pouch: Washing Buffer	PBS with 0.05% Tween20, bring to 1 liter with distilled water and store refrigerated.

### **MATERIALS REQUIRED BUT NOT PROVIDED**

Variable single and multichannel pipettors with appropriate tips  
Dilution tubes  
Distilled or deionized water

Wash bottle  
Timer  
Absorbent paper towels  
Microplate reader with 450nm filter

### **PRECAUTIONS**

1. Bring all reagents to room temperature (19° - 27°C) before use.
2. Store reagents at 2 to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
4. Adhere to all time and temperature conditions stated in the procedure.
5. Never pipette reagents or samples by mouth.
6. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
7. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with aflatoxin. Wear protective gloves and safety glasses when using this kit.
8. Dispose of all materials, containers and devices in the appropriate receptacle after use.

### **ASSAY PROCEDURE**

1. Bring all the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. Q.S. to 1 Liter with distilled water and store refrigerated when not in use.
2. Remove any debris or precipitate from the urine sample by filtration or centrifugation.
3. Dilute an aliquot of both the urine standards and samples 1:20 with distilled water e.g. 50 µl plus 900 µl distilled water.
4. Place one mixing well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microtiter Wells in another microwell holder.
5. Dispense 200 µl of the assay buffer into each mixing well.
6. Using a new pipette tip for each, add 100 µl of each diluted standard and sample to the appropriate mixing well containing the assay buffer. Mix by priming pipettor at least 3 times.
7. Using a new pipette tip for each, transfer 100 µl of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 1 hour. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.

8. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS-Tween wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 3 washes.
9. Tap the microwells (face down) on a layer of absorbent towels to remove residual water.
10. Add 100  $\mu$ l of conjugate to each antibody coated well and incubate at ambient temperature for 15 minutes.
11. Repeat steps 6 and 7.
12. Measure the required volume of Substrate Reagent (1 ml/strip or 120  $\mu$ l/well) and place in a separate container. Add 100  $\mu$ l to each microwell. Incubate at room temperature for 15 minutes.
13. Measure the required volume of Stop Solution (1 ml/strip or 120  $\mu$ l/well) and place in a separate container. Add 100  $\mu$ l in the same sequence and at the same pace as the Substrate was added.
14. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450 nm filter. Record the optical density (OD) of each microwell.

Note: If more than two strips are used in an assay, the use of a multichannel pipette is recommended for all additions in order to mitigate “beginning to end” variation.

### **INTERPRETATION OF RESULTS**

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0.0) standard against the aflatoxin content of the standard. Unknowns are measured by interpolation from the standard curve.

If a sample gives an OD less than the highest standard it should be further diluted in distilled water and re-tested. The extra dilution should be taken into account when calculating the result. Due to the nature of inhibition immunoassays, values derived by extrapolation outside of the measured highest and lowest standards are likely to be erroneous.

### **Assay Characteristics**

Data from ten consecutive standard curves gave the following results:

Ppb (ng/ml)	B/B <sub>0</sub>	%CV
0.0	100	-
0.15		
0.40		
0.80		
1.50		
4.00		

—

## SELECTED REFERENCES

- <sup>1</sup> Wogan, Gerald N., Aflatoxins as Risk Factors for Hepatocellular Carcinoma in Humans. *Cancer Research* 1992; 52: 2114s-2118s
- <sup>2</sup> Williams, Jonathan, et. al., Human Aflatoxicosis in Developing Countries: A review of Toxicology, Exposure, Potential Health Consequences, and Interventions. *American Journal Clinical Nutrition* 2004; 80: 1106-22
- <sup>3</sup> Krishnamachari, K.A.V.R, et. al., Hepatitis Due to Aflatoxicosis: An Outbreak in Western India. *Lancet* 1975; 1061-1063
- <sup>4</sup> Azziz-Baumgartner, et. al., Case-Control Study of an Acute Aflatoxicosis Outbreak, Kenya 2004. *Environmental Health Perspectives* 2005; 113: 1779-1783
- <sup>5</sup> Newman, Shelley Joy, et. al., Aflatoxicosis in nine dogs after exposure to contaminated commercial dog food. *J Vet Diagn Invest* 2007; 19: 168-175
- <sup>6</sup> Kussak, Anders, et. al., Determination of Aflatoxins in Dust and Urine by Liquid Chromatography / Electrospray Ionization Tandem Mass Spectrometry. *Rapid Communications in Mass Spectrometry* 1995; 9: 1234-1237
- <sup>7</sup> R.A. Zilinskas, Iraq's biological weapons. The past as future? *J. Am. Med. Assoc.* 1997; 278: 418-424
- <sup>8</sup> Zhu, Jia-qi, et. al., Correlation of Dietary Aflatoxin B1 Levels with Excretion of Aflatoxin M1 in Human Urine. *Cancer Research* 1987; 47: 1848-1852
- <sup>9</sup> Qian, Geng-Sun, et. al., A Follow-up Study of Urinary Markers of Aflatoxin Exposure and Liver Cancer Risk in Shanghai, People's Republic of China. *Cancer Epidemiology, Biomarkers & Prevention* 1994; 3: 3-10