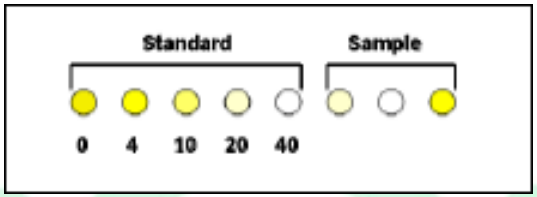
Aflatoxin ELISA Test Kit

Qualitative Result

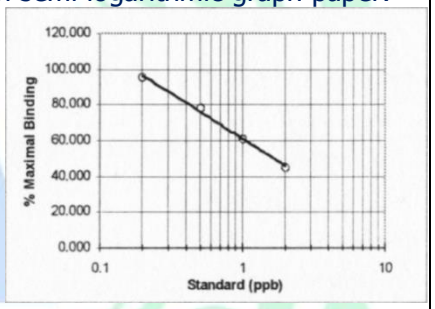
Qualitative result can be derived by visual comparison of the sample absorbance to the absorbance of the standard well.



Thus if the resilt of color reaction in sample is equal Standard 0 ppb means do not have aflatoxin in the sample but if the result of color reaction in sample is less color than Standard 0 ppb means have aflatoxin in the sample and can told that more or less Standard 4, 10, 20, 40 ppb by comparison the color of standard.

Quantitative Result

Quantitative interpretation base on the absorbance of standard aflatoxin in the test to establish standard curve. Plot standard curve on semi logarlthmic graph paper.



1. Calculate the mean value of absorbance values obtained for the standards and the samples (B) and divided by the absorbance value of zero standard (B_0)
2. The absorbance values of other standards and sample are calculated in percentage of absorbance (% maximal binding)

   $$\% \text{ maximal binding} = \frac{B}{B_0} \times 100$$

3. Construct the standard curve using % maximum binding (B/B_0) of each standards plotted on semiloganthmic graph paper. Each Aflatoxin B_1 standard concentration is plotted on X-axis and % maximum binding in on Y-axis.
4. The Aflatoxin B_1 concentration of each sample can be read from the standard by plotting % maximum binding on Y-axis. Then make the straight line against the standard curve and make the straight line downward to X-axis. In order to obtain the Aflatoxin B_1 concentration in ng./g. actually contained in a sample, the concentration read from standard curve must be multiplied by 20 (dilution factor)

Remark: ELISA method was the Screening test, positive sample recommenced to be confirm by Confirmation Method such as LC/MS etc.

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Aflatoxin ELISA Test Kit

Introduction

Aflatoxins are a chemically diverse group of compounds product as secondary metabolites of certain strains of *Aspergillus flavas, A. parasiticus* and *A. nomius* that may commonly in/on food and feedstuffs. The major aflatoxins of concem are Aflatoxin B_1, B_2, G_1 and G_2. Aflatoxin B_1 is the most toxic compound in this series, has been found to be one of the most potent liver carcinigents occurring naturally. The EU. Maximum levels (MLs) for a number of Aflatoxins have been set by Commission Regulation (EC) No 165/2010. Consequently, many laboratories ars looking for the analyses method with a highly sensitive and speccific for detecting aflatoxin. The method are so-called Enzyme-Linked Immunosorbent Assay (ELISA) by name ScreenEZ®

Principle of ScreenEZ® Aflatoxin ELISA Test Kit

Aflatoxin kit is a direct competitive Enzyme-Linked Immunosorbent Assay. An antibody with a high alfinity for Aflatoxin B_1 is coated onto polystyrene microwells. Aflatoxin is extracted from a ground sample by shaking with water/methanol solution. Free toxin in the sample and standards is added to the appropriate well and is allowed to compete with enzyme-linked toxin (conjugate) for the antibody binding site. After the particular incubation time, the content of the wells are removed and wash to remove any unbound toxin or enzyme-labeled toxin. A substrate is then added which reactswith the bound enzyme conjugate to produce blue color. The intensity of the color is directly proportional to amount of bound conjugate and inversely proportional to the amount of Aflatoxin B_1 in the standard or sample. Therefore, as the concentration of Aflatoxin B_1 in the sample or standard increases, the intensity of the blue color will decrease.

Specificity

Aflatoxin B_1	100.0 %
Aflatoxin B_2	21.4 %
Aflatoxin G_1	25.0 %
Aflatoxin G_2	2.5 %

Limit of Detection

The mean lower detection limit of ScreenEZ® Aflatoxin ELISA Test Kit is 0.4 ppb

Components of ScreenEZ® Aflatoxin ELISA Test Kit

Microtiter Plate-antibody coated (12×8 wells)	1 plate
AFB_1 standards (0, 0.2, 0.5, 1, 2 ng./ml. (ppb))	1 vials
AFB_1-HRP Conjugate (100 µl/vial)	6 vials
Supstrate (12 ml)	1 vials
Stop Solution (12 ml)	1 vials (Orange dot)
Conjugate Buffer (8 ml)	1 vials (Green dot)
Washing Buffer (10x, 60 ml)	1 vials (Blue dot)

Preparation of the sample

Weigh 20 glams of ground sample

Method 1 Flask → Add 100 ml. of 70% methanol then seal flask with parafilm → Using shaker at 300 rpm for 30 minutes

Method 2 Blender → Add 100 ml. of 70% methanol into blender → Using high speed blend for 2-3 minutes

Method 3 Bottle → Add 100 ml. of 70% methanol then properly cap → Shake by hand for 5 minutes or Vortex for 2-3 minutes

Stand for 5-10 minutes to allow some setting of the slurry

Filter the extract though a Whatman No. 4 filter

Collect the extract into a clean container (Ratio = 1 : 5)

Diluted the filtrate with diluted Washing buffer (0.01M PBS-T)
(1 ml. filtrate + 3 ml. diluted Washing buffer (0.01M PBS-T)) (Ratio = 1 : 4)

Ready for ELISA test

Remark: Users can select the appropriate exteaction method

Warning and Precaution for users

- Bring all reagents to room temperature before use.
- Store reagents at 2-8 °C. Never freeze the kit component, except AFB_1-HRP conjugate.
- Do not return unused reagent back into their original bottles.
- Do not allow stop solution and substrate to contract skin or eyes, if exposed, flush with water.
- Dispose of all materials containers and devices in an appropriate recptacle after use.

Preparation of solution prior to use

Microtiter plate (Ready to use)
Return unused strip into resealable bag with desiccant and store at 2-8 °C

Aflatoxin standard (Ready to use)
The Aflatoxin standard (AFB_1) solutions contain 0, 0.2, 0.5, 1 and 2 ng./ml. (ppb). Keep in the dark.

Enzyme Conjugate
Reconstitution the vial of Conjugate (AFB1-HRP) with 1 ml. conjugate buffer per vial, mix thoroughly. Keep conjugate solution at 2-8 °C (for longer shelf-life, recommence to store in a freezer -20 °C

Substrate (Ready to use)
Bring to room temperature before use. Avoid direct (sun) light.

Stop solution (Ready to use)
The stop solution contains 0.3M Phosphoric acid (H_3PO_4). Do not allow the reagent to get into contact with skin and/or eyes.

Conjugate Buffer (Ready yo use)
For reconstitution of the Enzyme Conjugate. The buffer stored at 2-8 °C

Washing buffer (10x)
Before use, should be diluted with 540 ml. of distilled water to make 0.01M PBS-T. Use for diluted filtrate extraction and washing micro well.

Assay procedures

1. Add 50 µl of AFB_1 standard into the well.
2. Add 50 µl sample into the well according to the design.
3. Add 50 µl enzyme conjugate (AFB_1-HRP) into every well.
4. Incubate at room temperature 37 °C in the dark for 30 minutes.
5. Discard the solution of the well and wash 3 times with Washing buffer.
6. Add 100 µl of Substrate into the well.
7. Incubate at room temperature 37 °C in the dark for 5-10 minutes.
8. Stop the reaction by adding 100 µl of Stop solution.
9. Read the optical density (OD) of each microwell with ELISA Reader at wavelength 450 nm. (Quantitative result). Read aflatoxin concentration in ppb (µg./kg.)