Total Aflatoxin
ELISA Kit

ZellBio GmbH (Germany)

Cat. No：ZB-15144C-H9648

Sample Types Validated
Edible oil, Feedstuff, Feed, Grain, Milk, Powder Milk

For Research Use Only. Not For In Vitro Diagnosis

Please read this insert completely prior to using the product.

Important note:

Only the brochure inside the kit should be considered for the assay procedure, as it is the latest version of the insert.

ZellBio GmbH assay kit is used to **quantitative** assay **Total Aflatoxin** on the basis of the Biotin double antibody sandwich technology. **ZellBio GmbH** ELISA kit takes **one-step** method with which solutions need minimum diluting, because we simplify the dilute process by our lab techniques. This kit is for research only and is not for use in diagnostic procedures.

Intended Use

ZellBio GmbH Total Aflatoxin in the sample of Edible oil, Feedstuff, Feed, Grain, Milk, Powder Milk.

Test principle

ZellBio GmbH assay kit uses enzyme-linked immunosorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the **Total Aflatoxin.** Add Total Aflatoxin protein to the wells, which are pre-coated with anti-Total Aflatoxin monoclonal antibodies and after that, add anti-Total Aflatoxin antibodies labeled with biotin to combine with streptavidin-HRP, which forms an immune complex. Remove unbound enzymes after incubation and washing. Add substrate.

Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Total Aflatoxin are positively correlated.

Materials supplied in the Test Kit

	Materials	96 Tests	48 Tests
1	Standard (6400pg/mL)	0.35ml	0.35ml
2	Standard diluent	3ml	3ml
3	Microplate	12×8	6×8
4	Strp-HRP-Conjugate Reagent	6ml	3ml
5	Wash Solution (30X)	20ml	20ml
6	Biotin- Total Aflatoxin -Ab	1ml	0.5ml
7	Chromogen Solution	12ml	6ml
8	Stop Solution	6ml	3ml
9	Instruction	1	1
10	Adhesive Sheet	2	2

Materials required but not supplied

1. 37℃ incubator
2. Standard Microplate reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for Standards and samples dilution
6. Absorbent paper
7. Mortar or laboratory mill
8. Laboratory scales
9. Erlenmeyer flask or plastic centrifuge tube
10. 100mL measuring cylinder
11. Funnel
12. Filter (e.g. Whatman No. 1)
13. Collection containers for sample extracts
14. Containers for diluted sample extracts, e.g. 1mL volume
15. Microplate washer or multi-channel pipette for washing
16. Methanol
17. Distilled water
18. Normal saline (NaCl 0.9%)

Important Notes

1. Before opening the kit kept at the temperature of 2-8℃, it takes at least 30 minutes to increase naturally to room temperature. After

breaking the seal of ELISA coated-plate, some of the stripes used should be kept in a hermetic bag.

2. When adding samples, sample injector must be used for each time and should also be frequently checked for its precision to avoid individual error.

3. The instruction must be strictly followed while the reading of ELISA reader must be set as the standard of determining the experiment result.

4. Pipette tips and seal plate membrane in hand should not be used more than once in order to avoid cross contamination.

5. All samples, washing concentration and wastes of every kind should be disposed of as an infective agent.

6. Other reagents not needed must be packed or covered. Reagents of different batches must not be mixed and should be used before their respective validity dates.

7. Substrate is sensitive to light and therefore should not be exposed to light for too long.

Washing method

Preparation of 1X Wash Buffer: Dilute 30X wash buffer with distilled water. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Manual washing method: Washing by hand: Shake off the liquids in the wells of the ELISA plate; Lay several bibulous papers on the test bed and pat hard the ELISA plate several times downward; then inject at least 300μl of diluted washing concentration for 1-2 minutes' soaking. Repeat this process as needed.

Automatic washing method: Washing by automatic plate washer: If there is an automatic plate washer, it should only be used in the test when you are quite familiar with its functions.

Assay Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level **Total Aflatoxin** were tested 12 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level **Total Aflatoxin** were tested on 3 different plates, 10 replicates in each plate.

$$CV\ (\%) = (SD/mean) \times 100$$

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Sample preparation

- Solid samples should be present in crushed, homogeneous form as a fine to medium-fine powder. If necessary, the solid sample must be crushed in a mortar or with a laboratory mill.

- 5g of the crushed, homogeneous sample is weighed in a suitable container, such as an Erlenmeyer flask or a plastic centrifuge tube and

mixed with 25mL of methanol/water (70/30 v/v). This suspension is shaken intensively for 3 minutes to extract the Aflatoxin. The suspension is then filtered via a folded filter for quantitative analysis. It is recommended to let the solids shortly settle before filtering.

- The filtrate (sample extract) is diluted in a new container with a 1:10 ratio with Normal Saline.

1 part filtrate + 9 parts Normal Saline, e.g. 100 µL filtrate + 900 µL Normal Saline

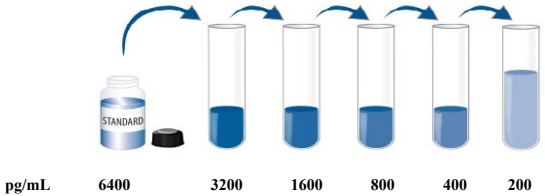
- The diluted filtrate can now be tested with the Total Aflatoxin ELISA Assay Kit. 1 mL of diluted filtrate equals 0.1 g solid sample = dilution factor 50.

* Note: if higher Aflatoxin concentrations are expected, then the filtrate must be diluted at a higher ratio than 1:10, while the ratio of the methanol/water mixture (70/30, v/v) must be maintained.

Assay procedure

1. Dilution of standard solutions: This kit has a standard of original concentration, which could be diluted in small tubes by user independently following the instruction:

6400pg/mL	Standard No.6	60μl Original Standard
3200pg/mL	Standard No.5	60μl Original Standard + 60μl Standard diluents
1600pg/mL	Standard No.4	60μl Standard No.5 + 60μl Standard diluents
800pg/mL	Standard No.3	60μl Standard No.4 + 60μl Standard diluent
400pg/mL	Standard No.2	60μl Standard No.3 + 60μl Standard diluent
200pg/mL	Standard No.1	60μl Standard No.2 + 60μl Standard diluent
0pg/mL	Standard No.0	60μl Standard diluent



2. The number of stripes needed is determined by that of samples to be tested and added by that of standards. It is suggested that each standard solution and the blank well should be arranged with duplicates as much as possible.

3. Sample injection: a) Blank well: no sample, anti Total Aflatoxin antibody labeled with biotin or streptavidin-HRP is added to

comparison blank well except Chromogen solution and stop solution while taking the same steps that follow. b) Standard solution well: Add 50μl standard and streptavidin-HRP 50μl (biotin antibodies have united in advance in the standard so no biotin antibodies are added). c) Sample well to be tested: Add 40μl sample and then 10μl Total Aflatoxin antibodies, 50μl streptavidin-HRP. Then cover it with a seal plate membrane. Shake gently to mix them up. Incubate at 37℃ for 60 minutes.

4. Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

5. Washing: Remove the seal plate membrane carefully, drain the liquid and shake off the remaining liquid. Fill each well with a washing solution. Drain the liquid after 30 seconds' standing. Then repeat this procedure four times and blot the plate.

6. Color development: Add 100μl Chromogen solution to each well as well. Incubate for 10-20 minutes at 37℃ away from light for color development.

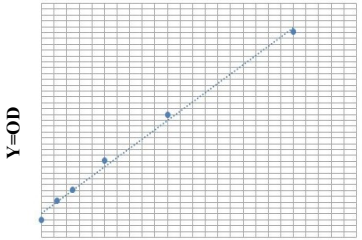
7. Stop: Add 50μl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).

8. Reading: Take a blank well as zero, measure the absorbance (OD) of each well one by one under 450 nm wavelength, which should be carried out within the 10 minutes after having added the stop solution.

9. According to standards' concentrations and the corresponding OD values, Draw the standard curve using point to point calculation mode. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Special software could be employed to calculate as well.

Calculation

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the coordinate paper. According to the OD value of the sample, locate its corresponding concentration; point to point or calculate the linear regression equation of standard curve according to the concentration of the standards and the OD value. Then substitute with the OD value of the samples to calculate their concentration.



X= Total Aflatoxin Concentration

A typical standard curve of ZellBio GmbH **Total Aflatoxin** Assay kit
(The chart is indicative only)

Assay range：200pg/mL →6400pg/mL

Sensitivity：25pg/mL

Summary

Prepare reagents, samples and the standards

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Add (40µl sample(s) +10µl Total Aflatoxin -Ab), 50µl standards and 50µl Streptavidin-HRP, Let them react for 60 minutes at 37℃

⇓

Wash the plate five times with 300µl diluted wash buffer

⇓

Add 100µl Chromogen solution. Incubate for 10-20 minutes at 37℃ for color development

⇓

Add 50µl stop solution

⇓

Read the OD value within 10min at 450 nm

⇓

Calculation

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