



-Operation Manual of the kit for Detection of Aflatoxin B<sub>1</sub>

Based on the principle of solid enzyme-linked immunosorbent assay, the kits can be used to detect aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) by direct competitive immunosorbent reactions between anti-AFB<sub>1</sub> antibody and antigen-enzyme conjugate or tested antigen, combining with catalytic color reaction of the enzyme. The kits are characterized by high sensitivity, good specificity and easy to operate and read out.

1. Composition of the reagent kit:

All reagents required for the enzyme immunoassay (including standards) are contained in the test kit.

We provide three specifications of kits: 12-wells, 24-wells and 48-wells.

Each test kit contains:

- |  |                    |
|--|--------------------|
| 1. antibody-coated microplate:   | 12, 24 or 48 wells |
| 2. reagent A: sample diluent   | white cap          |
| 3. reagent B: AFB <sub>1</sub> standard solutions<br>(0.1, 0.5, 1, 5, 10, ng/ml) | red cap            |
| 4. reagent C: antigen-enzyme conjugate   | glass tubes        |
| 5. reagent D: diluent for antigen-enzyme conjugate                               | green cap          |
| 6. reagent E: concentrated washing solution (300*)                               | white cap          |
| 7. reagent F: substrate solution A   | blue cap           |
| 8. reagent G: substrate solution B   | black cap          |
| 9. reagent H: stopping solution  | yellow cap         |
| 10. microplate holder:   | penetrate          |



2. Preparation of samples:

Food

2.1.1 Solid sample with low fat

Weigh 5.0g of sample in a 50ml ground-grass tube (sample must be pulverized into 20 mesh or porphyzied homogeneously). The sample is extracted with 25ml extractant of methanol-water (1:1). Plug the tube and snake it for 5-10 minutes. The extract is then filtered, discard 1/4 preliminary filtrate, the other is diluted with reagent A according to the following table:

sample	filtrated solution (ml)	reagent A (ml)	diluted rate
fermented bean foods,			
other grains	0.1	0	0
rice, edible oil	0.1	0.1	1:1
maize for food	0.05	0.15	1:3

2.1.2 Soy sauce, vinegar:

Weigh 5.0g of sample in a 25ml beaker, transfer sample with 5ml distilled water into 125ml separatory funnel, add 20ml chloroform ,plug the funnel and shake it for 3 minutes. Let the chloroform out of the funnel when it is stationary and layered. Filter the extract with a slow filter paper filled with 5g  $\text{Na}_2\text{SO}_4$  into a 50ml-evaporating dish. Shake and extract once more with another 5ml chloroform. The chloroform layer is also filtered into the evaporating dish. Wash the filter paper with a small amount of chloroform, put the eluent into the dish, and then make the evaporating dish dry on water bath of  $65^\circ\text{C}$  under ventilating condition in a ventilating closet. Afterwards, the test portion is dissolved with 25.0ml extractant and diluted with reagent A as the above table.



### 2.1.3 Edible oils

Weigh 5.0g of sample in a small beaker, transfer the test sample into a 125ml separatory funnel with 20ml hexane or petroleum ether. Wash the small beaker several times with 25.0ml extractant of methanol-water (1:1), the washing solution is also transferred into the funnel. Shake the funnel for 5 minutes. Let the lower extract layer out of the funnel when it is stationary and layered. Afterwards, extractant is diluted with reagent A as the above table.

### 2.1.3 Wine

Weigh 5.0g of wine in a small beaker, transfer the test sample into a 125ml separatory funnel with 20ml hexane or petroleum ether. Wash the small beaker several times with 25.0ml extractant of methanol-water (1:1). The washing solution is also transferred into the funnel. Shake the funnel for 5 minutes. Let the lower extract layer out of the funnel when it is stationary and layered. Filter the extract with a slow filter paper filled with 5g  $\text{Na}_2\text{SO}_4$  into a 50ml-evaporating dish. Shake and extract once more with another 5ml chloroform. The chloroform layer is also filtered into the evaporating dish. Wash the filter paper with a small amount of chloroform, put the eluent into the dish, and then make the evaporating dish dry on water bath of 65°C under ventilating condition in a ventilating closet. Afterwards, the test portion is dissolved with 25.0ml extractant and diluted with reagent A as the above table.

### 2.1.4 Beer

Pipette 5.0ml sample in a 25ml volumetric flask, add 12.5ml methanol, scale up the volume to 25ml with distilled water, shake it for 10 minutes, then use it directly in the test.

### 2.1.5 Peanuts

Comminute samples, weigh 5.0g in a 50ml ground-grass tube, add 25.0ml extractant of



methanol-water (1:1) and 20ml hexane or petroleum ether, shake it for 10 minutes, filter it into separatory funnel, let the lower extract layer out of the funnel when it is stationary and layered.

Afterwards, extractant is diluted with reagent A as the above table.

#### 2.1.6 Cake and peanut butter etc.

Weigh 5.0g in a 50ml ground-glass tube, add 25.0ml extractant of methanol-water (1:1) and 20ml hexane or petroleum ether, shake it for 10 minutes, filter it into separatory funnel, let the lower extract layer out of the funnel when it is stationary and layered into a beaker.

Pipette 10.0ml extract (equal to 2.0g samples) in 125ml separatory funnel,

##### 1. Preparation of reagents:

(1) Each vial of reagent C is added with 1.5ml reagent D, dissolved and mixed. It can be stored for use within 6 months at 2-8°C.

(2) Adding 300ml distilled water into reagent E makes up the washing solution.

(3) Let the reagent kit to be at room temperature.

(4) Wash the microplate twice with reagent E at an interval of 1 minute. Notice that the washing solution shouldn't be overflow and the microplate should be patted to be dry on a water-absorbing paper.

### 三 Test procedure

#### 1. Number the microplate wells:

No. 1—3 are wells for standard control test. No. 4—12 are sample wells.

#### 2. Inject the wells with reactants:

Prepared solutions and tested sample diluents are injected into the wells in an orderly way as indicated in the following table.



Order	Volume to be added	Well number											
		1	2	3	4	5	6	7	8	9	10	11	12
1	50 $\mu$ l	A	B	A	diluted sample solution								
2		Shake											
3	50 $\mu$ l	D	C	C	C	C	C	C	C	C	C	C	C
4		Shake											

3. Reaction:

Incubated at 37°C for 30 minutes after the above addition of reagents.

4. Washing :

Take out the microplate and wash it five times with washing solution E at intervals of 2 minutes.

Notice that the washing solution should not be over flow and the microplate should be patted to be dry on a water-absorbing paper after washing.

5. Coloration

Add 50  $\mu$ l substrate solution A and 50  $\mu$ l substrate solution B into each well of the microplate.

Shake and then incubated at 37°C for 15 minutes for visual estimation.

6. Stopping:

Add 50  $\mu$ l stopping solution (reagent H) into each well to stop reactions for instrumental detection.

7. Detection:

Compare colors of well 1—3 .If the color of well 3 is the deepest, followed by well 2, and the color of well 1 is nearly colorless, it demonstrates that the standard control is correct.

(1) Visual estimation: (visual before the adding of stopping solution )



Compare color of sample well with that of the well 2. If the color of the sample well is not deeper than that of the well 2, it is positive. Conversely ,it is negative. If the colors of the two wells are approximately the same, then it is necessary to detect it by instrument method or by normal international test method.

(2) Instrumental detection:

Determine value A at 450nm with the instrument. It is positive if  $A_{\text{sample}} \leq A_{\text{well 2}}$  , and it is negative if  $A_{\text{sample}} > A_{\text{well 2}}$  .

8. Semi-quantitative of the test:

If the detected sample shows positive, then the content of aft.B<sub>1</sub> can be estimated by the following table:

Dillution rate	content of aft. B <sub>1</sub> (ppb)
0	>5
1:1	>10
1:3	>20

9. Quantitative determination by instrument:

(1) standard curve method<sup>1</sup>

The concentration of aft B1standard solution are 0,0.01,0.1,1,5,10,20μg/kg, respectively, the absorbance of each wells are measured photometrically at 450nm, then draw standard curve.

The absorbance value of standards (or samples) are divided by the absorbance value of the zero standard and multiplied by 100. The absorbance value of the zero standard is thus

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<sup>1</sup> <sup>1</sup> Please tell us when you want to draw standard curve, because we will prepare 50μg/kg standard solution for you!



made equal to 100% and the absorbance values are quoted in percentage.

$$\times 100\% = \% \text{ Absorbance}$$

The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the aft B1 concentration.

The aft B1 concentration in  $\mu\text{g}/\text{kg}$  corresponding to the extinction of each sample can be read directly from standard curve. Then the content of sample is calculated according to the following formula:

$$C_{\text{sample}} (\mu\text{g}/\text{kg}) = \frac{C \times V \times D}{m} \dots\dots\dots (1)$$

Where:

C— concentration read from the standard curve

V— volume of extractant

D— dilution rate

$$\frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \quad m \text{— weight of sample}$$

(3) Experience formula method

Regent B is diluted by regent A at 10 rate. The diluent is new standard solution (0.1 $\mu\text{g}/\text{kg}$ ). Add 50 $\mu\text{l}$  new standard solution to the 3<sup>rd</sup> well, test its absorbance, then the content of aft B1 in the sample is calculated according to the following formula:

$$\text{Lgx} = \frac{A_{\text{No.3}} - A_{\text{sample}}}{A_{\text{No.3}} - A_{\text{No.2}}} - 1 \dots\dots\dots (2)$$

Substitute the value of “x” for the “x” of following formula:



$$C_{\text{sample}} (\mu\text{g}/\text{kg}) = \frac{X \times V \times D}{m} \dots\dots\dots (3)$$

Where:

V, D, m are as the same as formula (1)

A<sub>No.3</sub>— absorbance of 3<sup>rd</sup> well (0.1μg/kg standard solution)

A<sub>No.2</sub>— absorbance of 2<sup>nd</sup> well (1μg/kg standard solution)

A<sub>sample</sub>— absorbance of sample well

四. Storage of the kits:

1. The kits should be stored at 2-8°C. They mustn't be freezing-stored below 0°C.
2. Term of validity: 6 months.

Notice that once antigen-enzyme conjugate (reagent C) is opened and diluted with reagent D ,it must be stored at 2-8°C and must be weed up within 6 months.

五. Standards of permissible aft. B<sub>1</sub> contents in some commodity samples (GB26 71-81,GB 8381-87) .

sample	permissible aft. B <sub>1</sub> content (ppb)
milk substitutes for babies	must not be detected
grains, beans, fermented foods	≤5
rice, edible oils	≤10
maize for food	≤20

六. Cautions

1. In order to avoiding pollution, containers and pipet tips contaminated with reagent B or reagent C must be immersed in 5% NaClO solution for



one day, and then washed for another use.

2. Reagents with different lot number must not be used at the same time.

七. Exp

八. Add: 7 Qiangrong Road, Wuxi, 214063, P.R. China

Tel: 0086-510-5517261 5514690

Fax: 0086-510-5500928

Post code : 214063

### Operation Manual of the kit for Detection of Aflatoxin B<sub>1</sub>

(For Use in Feed Inspection)

Based on the principle of solid enzyme-linked immunosorbent assay, the kits can be used to detect aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) through competitive immunosorbent reactions between anti-AFB<sub>1</sub> antibody and antigen-enzyme conjugate or tested antigen, combining with catalytic color reaction of the enzyme. The kits are characterized by high sensitivity, good specificity and easy



to operate and read out.

一、 Composition of the reagent kit:

1. antibody-coated microplate:	48 wells
2.reagent A: sample diluent	1 vial
3. reagent B: AFB <sub>1</sub> standard solution	1 vial
4. reagent C: antigen-enzyme conjugate	2 vial
5. reagent D: diluent for antigen-enzyme conjugate	1 vial
6. reagent E: concentrated washing solution (300*)	1 vial
7. reagent F: substrate solution A	1 vial
8. reagent G: substrate solution B	1 vial
9. reagent H: stopping solution	1 vial
10. microplate holder:	1 plate

二、 Preparations for test:

1. sample treatment:

weight 5g sample into a 50ml ground-grasstube (solid sample must be pulverized into 20 mesh or porphyzied homogeneously).The sample is extracted with 25ml extractant of methanol-water (7:3). Plug the tube and snake it for 5-10 minutes. The extract is then filtered, and the filtrate is diluted with reagent A according to the following table:

sample	filtrated solution (ml)	reagent A (ml)	diluted rate
broiler, growing chicken feeds	0.1	0.1	1:1
mixed or formula feeds	0.05	0.15	1:3
maize for feed, peanut solvent	0.05	0.45	1:9



2. Preparation of reagents:

(1) Each vial of reagent C is added with 1.5ml reagent D, dissolved and mixed. It can be stored for use within 6 months at 2-8°C.

(2) Adding 300ml distilled water into reagent E makes up the washing solution.

(3) Let the reagent kit to be at room temperature.

(4) Wash the microplate twice with reagent E at an interval of 1 minute. Notice that the washing solution shouldn't be overflow and the microplate should be patted to be dry on a water-absorbing paper.

三 Test procedure

1. Number the microplate wells:

No. 1—3 are wells for standard control test. No. 4—12 are sample wells.

2. Inject the wells with reactants:

Prepared solutions and tested sample diluents are injected into the wells in an orderly way as indicated in the following table.

order	volume to be added	well number											
		1	2	3	4	5	6	7	8	9	10	11	12
1	50 $\mu$ l	A	B	A									
2													
3	50 $\mu$ l	D	C	C	C	C	C	C	C	C	C	C	C
4													

3. Reaction:

Incubated at 37°C for 30 minutes after the above addition of reagents.



4. Washing :

Take out the microplate and wash it five times with washing solution E at intervals of 2 minutes.

Notice that the washing solution should not be over flow and the microplate should be patted to be dry on a water-absorbing paper after washing.

5. Coloration

Add 50  $\mu$ l substrate solution A and 50  $\mu$ l substrate solution B into each well of the microplate.

Shake and then incubated at 37°C for 15 minutes for visual estimation.

6. Stopping:

Add 50  $\mu$ l stopping solution (reagent H) into each well to stop reactions for instrumental detection.

7. Detection:

Compare colors of well 1—3. If the color of well 3 is the deepest, followed by well 2, and the color of well 1 is nearly colorless, it demonstrates that the standard control is correct.

(1) Visual estimation: (visual before the adding of stopping solution )

Compare color of sample well with that of the well 2. If the color of the sample well is not deeper than that of the well 2, it is positive. Conversely, it is negative. If the colors of the two wells are approximately the same, then it is necessary to detect it by instrument method or by normal international test method.

(2) Instrumental detection:

Determine value A at 450nm with the instrument. It is positive if  $A_{\text{sample}} \leq A_{\text{well 2}}$  ,

and it is negative if  $A_{\text{sample}} > A_{\text{well 2}}$  .

8. Semi-quantitative of the test:



If the detected sample shows positive, then the content of aft.B<sub>1</sub> can be estimated by the

following table:

Dillution rate	content of aft. B <sub>1</sub> (ppb)
1:1	>10
1:3	>20
1:9	>50

9. Quantitative determination by instrument:

(1) standard curve method<sup>2</sup>

The concentration of aft B<sub>1</sub> standard solution are 0,0.01,0.1,1,5,10,20µg/kg, respectively, the absorbance of each wells are measured photometrically at 450nm, then draw standard curve.

The absorbance value of standards (or samples) are divided by the absorbance value of the zero standard and multiplied by 100. The absorbance value of the zero standard is thus made equal to 100% and the absorbance values are quoted in percentage.

$$\frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100\% = \% \text{ Absorbance}$$

The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the aft B<sub>1</sub> concentration.

The aft B<sub>1</sub> concentration in µg/kg corresponding to the extinction of each sample can be read directly from standard curve. Then the content of sample is calculated according to the following formula:

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<sup>2</sup> Please tell us when you want to draw standard curve, because we will prepare 50µg/kg standard solution for you!



$$C_{\text{sample}} (\mu\text{g}/\text{kg}) = \frac{C \times V \times D}{m} \dots\dots\dots (1)$$

Where:

C— concentration read from the standard curve

V— volume of extractant

D— dilution rate

m— weight of sample

(4) Experience formula method

Regent B is diluted by regent A at 10 rate. The diluent is new standard solution (0.1μg/kg). Add 50μl new standard solution to the 3<sup>rd</sup> well, test its absorbance, then the content of aft B1 in the sample is calculated according to the following formula:

$$\text{Lgx} = \frac{A_{\text{No.3}} - A_{\text{sample}}}{A_{\text{No.3}} - A_{\text{No.2}}} - 1 \dots\dots\dots (2)$$

Substitute the value of “x” for the “x” of following formula:

$$C_{\text{sample}} (\mu\text{g}/\text{kg}) = \frac{X \times V \times D}{m} \dots\dots\dots (3)$$

Where:

V, D, m are as the same as formula (1)

A<sub>No.3</sub>— absorbance of 3<sup>rd</sup> well (0.1μg/kg standard solution)

A<sub>No.2</sub>— absorbance of 2<sup>nd</sup> well (1μg/kg standard solution)

A<sub>sample</sub>— absorbance of sample well

四. Storage of the kits:



1. The kits should be stored at 2-8°C. They mustn't be freezing-stored below 0°C.

2. Term of validity: 6 months.

Notice that once antigen-enzyme conjugate (reagent C) is opened and diluted with reagent D, it must be stored at 2-8°C and must be used up within 6 months.

五. Standards of permissible afl. B<sub>1</sub> contents in some commodity samples (GB13078-91).

sample	permissible afl. B <sub>1</sub> content (ppb)
broiler, growing chicken feeds	≤10
mixed or formula feeds	≤20
maize for feed, peanut solvent	≤50

六. Cautions

1. In order to avoid pollution, containers and pipet tips contaminated with reagent B or reagent C must be immersed in 5% NaClO solution for one day, and then washed for another use.

2. Reagents with different lot number must not be used at the same time.

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Post code : 214063



Hello, here is our sorry to reply you so late. From your note, we know that you are student in Southern Yangtze University in Wuxi. We are glad to invite you to visit the institute, if possible.

The following is the English version of ELISA KIT FOR AFB<sub>1</sub>.If any question, phone me , please. My name is Azhao , my office phone: 0510-5500928 (am 8:00~pm 4:30), E-mail: [zhao946@yahoo.com](mailto:zhao946@yahoo.com). Best wishes!

