# $EAB_1$-95 AFLATOXIN MICROPLATE READER

# Usage manual


## Shanghai Qianjian Instrument Co.,Ltd

Contents

## Ⅰ Summary

$EAB_1$-95 Aflatoxin Microplate Reader is the new product that is researched by Jiangsu Microbiology Institute, Shanghai Feed Inspection and Supervisision Station and Shanghai Qianjian Instrument Company Limited, and is made by Shanghai Qianjian Instrument Company Limited .The instrument can determine aflatoxin ($AFB_1$) in sample. It is applicable to determine $AFB_1$ for quantitative and qualitative analysis in grain, food, feed, grease, milk product, herb, beverage, wine.

The instrument primarily is composed of the test kit and the reader. The test kit is based on competitive enzyme immunoassay and the reader is based on photometer.The instrument reaches to determine $AFB_1$ in fleetness, exactitude, quantitation and limited quantitation. Whole process can complete in an hour. The operation is simple, safety, dependable, accurately.

## Ⅱ Technique Parameter

A work condition:(1) voltage 220V±10%,50 Hz
(2) environment temperature 5~35 ℃
(3) relative humidity ≤ 80% RH

B measure scope: 0.00~10 μg/ kg

C wavelengh: 450nm ± 2 nm

D sample modal: 48 or 24 holes plate

E prepare time: 15 minutes

F test rate: 20 samples/ an hour

G sensitivity: ≤ 0.1 μg/ kg

H precision: ±0.1 μg/ kg

I light source: halogen-tungsten light 6V/12 W

J power: 37W

K size specification:350×270×210 mm

L weight: 9 kg

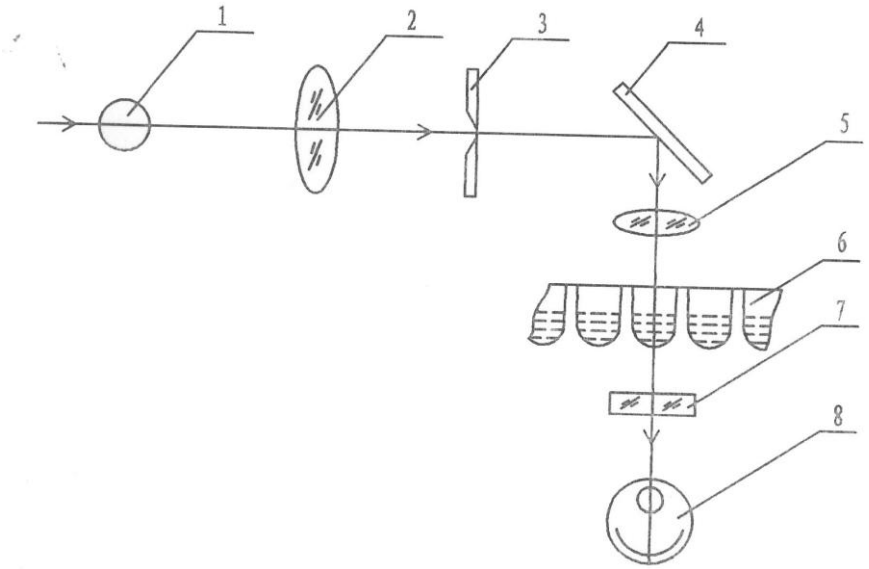
## Ⅲ Work Principle

The principle of $AFB_1$-95 Aflatoxin Microplate Reader is based on the competitive enzyme immunoassay and Beer-Lambert of photometer. The microtiter plates are coated with antibody against $AFB_1$.$AFB_1$-enzyme conjugate and the $AFB_1$ standards or $AFB_1$ in sample solution compete for $AFB_1$ antibody. Linked enzyme conjugate converts the colorless chromogen into a blue product. The stop reagent leads to the color changes from blue to yellow. The measurement is made photometically at 450nm.

During Measure, color chromogen in microliter plate absorbs light of particular wavelength. When wavelengths have an energy that is equivalent to the difference between the ground state and excited state energies, part of this wavelength will be absorbed. Convert the radiant energy into electrical signal through photo multiplier tube, and enlarge, show absorbance value (A) or transmittance (T) or concentration (C). The absorbance is inversely proportional to the $AFB_1$ concentration in the sample.

## Ⅳ Usage Elucidation

4.1 Main optical system in instrument




1 Light source 2 Focusing len 3 Grating 4 Mirror
5 Focusing len 6 Microliter plate 7 Filter 8 Photo multiplier tube

4.2 Instrument operation

4.2.1 Instrument preparation

4.2.1.1 Before using the instrument, first the user should understand the construction and work principle of the instrument and the function of the each operation knob. Check the instrumental safety, electrical wire should be firm and connect on earth

4.2.1.2 Turn on the power switch, adjust " T.A.C" choice knob to (T). There are three enlarging sensitivity ranks. The "1" rank is the lowest. Its operation method is to adopt the "1" rank possibly when it can adjust full degree in "100.0" to the blank in principle, in order to obtain the good stability. If the higher absorbance value of the blank, "1" rank can't satisfy the "100.0" full degree and may increase gradually rank.

4.2.1.3 Undraw the cover of the sample compartment, regulate the " T.0" knob and make figure manifestation as"00.0". Then push the cover of the sample compartment and make the photo multiplier tube suffer the light. Regulate the " T.100" knob. Make figure manifestation as "100.0". The instrument run runs for 15 minutes.

4.2.2 Reagent

4.2.2.1 Reagent kit components

(1) 1× Microtiter plats coated with antibody

(2) 1× A reagent: diluents

(3) 1× * B reagent: $AFB_1$ standard solution (1 μ g/ kg)

(4) 1× C reagent: peroxidase conjugated antigen

(5) 1× D reagent: conjugate diluents

(6) 1× E reagent: lavation (300 ×)

(7) 1× F reagent: substrate a

(8) 1× G reagent: substrate b

(9) 1× H reagent: stop solution

4.2.2.2 Preparation of samples

Weigh 5 g ground sample and transfer to 50 mL grass-stopper tube. Add 25 mL 50% methanol solution for the solid sample; Add 20 mL 50% methanol solution for liquid sample. Shake for 5~10 min. Filter the extract (the liquid sample do not need the percolation). Dilutes the filter with A reagent according to table1.

Table 1

| Sample | Filter (ml) | A reagent (ml) | Dilution factor |
|---|---|---|---|
| The food of bean ferment | 0.1 | 0 | 1 |
| Rice, edible oil, chicken , grow chicken feed | 0.1 | 0.1 | 2 |
| Corn (food),feed | 0.05 | 0.15 | 4 |
| Cake of corn (feed)and peanut | 0.02 | 0.18 | 10 |

4.2.2.3 Special sample Preparation

(a) High salt sample (sauce, decayed bean curd)

Weigh 5g ground samples and transfer to a separator. Add 6mL methanol and 10 mL $CHCl_3$. Shake for 2 min. Let layers separate completely. Drain $CHCl_3$ layer through filter paper in grass funnel to 50mL evaporator. Re-extract upper layers with 5mL $CHCl_3$ and shake, filter. Evaporate the combined $CHCl_3$ extract to near dryness at 65℃ in water baths. Add 25mL 50% methanol solution and dilute the solution with A reagent according to table1.

(b) Edible oils (the vegetable oil, salad oil, gingili oil...etc.)

Weigh 5g samples to the small beaker and transfer to 125mL separator with 20 mL n-hexane or petroleum ether. Wash the small beaker with 20 mL 50% methanol solution more times. Transfer washing to the separator. Shake for 2min. After separating layers drain the bottom layer. Re-extract upper layers with 5mL 50% methanol solution. Combine the bottom layer extract and turn into a volumetric flask, dilute to 250mL with 50% methanol solution. Again dilute the solution with A reagent, according to table1.

4.2.2.4 Reagents prepare

(a) Add 1.5 ml D reagent into C reagent, dissolve, mix, store for 6 months at 2～8℃.It is as enzyme conjugated antigen solution.

(b) Add 300 mL distilled water to E reagent. It is as lavation.

(c) Dilute B reagent with A reagent into 0.1 ng/ mL standard work solution B' reagent. The dilution factor is 10 (9:1).

4.2.3 Experiment step

(a) Bring all reagents to room temperature.

(b) Wash the wells of microtiter plats with E lavation for 2 times. Cannot overflow the lavation. After last wash tap wells hard enough on paper towel to remove remaining lavation.

(c) Mark serial number: No. 1～3 are as standard antitheses wells, No. 4～12 are as sample wells.

(d) Add reagents or sample solutions according to table 2

table 2

| order | Added volume | Well number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | 50μl | A | B | B’ | - - - - - - | | Sample solution | | | | | - - - - - - | |
| 2 | | | | | | | Shake | | | | | | |
| 3 | 50μl | D | C | C | C | C | C | C | C | C | C | C | C |
| 4 | | | | | | | Shake | | | | | | |

(e) Reaction: Incubate for 30 min at 37℃.

(f) Washing: dump the liquid out of wells into sink. Tap the microwell holder upside down onto a clean filter lower to remove all remaining liquid from the wells. Fill the wells with E lavation. Empty the wells again and remove all remaining liquid. Repeat the washing step five times.

(g) Show the color: Add 50 μL substrate a and substrate b to each well, shake. Incubate for 30 min at 37℃.Make preliminary observation and determination using visual method.

(h) Stop: Add 50 μL stop solution to each well. Make determination using the instrument method.

(i) Measurement: Compare the colors of No 1 ~3 wells, if No.3 well is the deepest, No.2 well is the second, No.1 well is colorless, explain that the standard is accurate.

4.2.4 Determination:

4.2.4.1 Put the microtiter plats into the sample compartment, check detector against the blank well, and press detector down. According to 4.2.1.3 step repeat to regulate for 2-3 times, then regulate" T, A, C" to " A" rank, regulate " A.O" knob, make showing value ".000".

4.24.2 One by one in order measure absorbance of standard wells and

sample wells, and record the " A" value.

4.2.5 Judgment of limited value

Compare " A" value of the sample wells with No.2 well, samples showing larger value than No.2 are negative, samples showing smaller value than No.2 are positive. Table 3 is $AFB_1$ contents when sample is positive.

Table 3

| Dilution factor | $AFB_1$ contents (μ g/ kg) |
|---|---|
| 1 | >5 |
| 2 | >10 |
| 4 | >20 |
| 10 | >50 |

4.2.6 Quantitation calculation

$$LgX=(Lg1-Lg0.1)\frac{A_3-As}{A_3-A_2}+Lg0.1=\frac{A_3-As}{A_3-A_2}-1$$

$$C=X\times\frac{V\times D}{m}$$

In equation:

X —$AFB_1$ concentration in dilution sample solution.

C —$AFB_1$ contents in sample (μ g/ kg).

$A_2$ —" A" value in standard B reagent well.

$A_3$ —" A" value in standard B' reagent well.

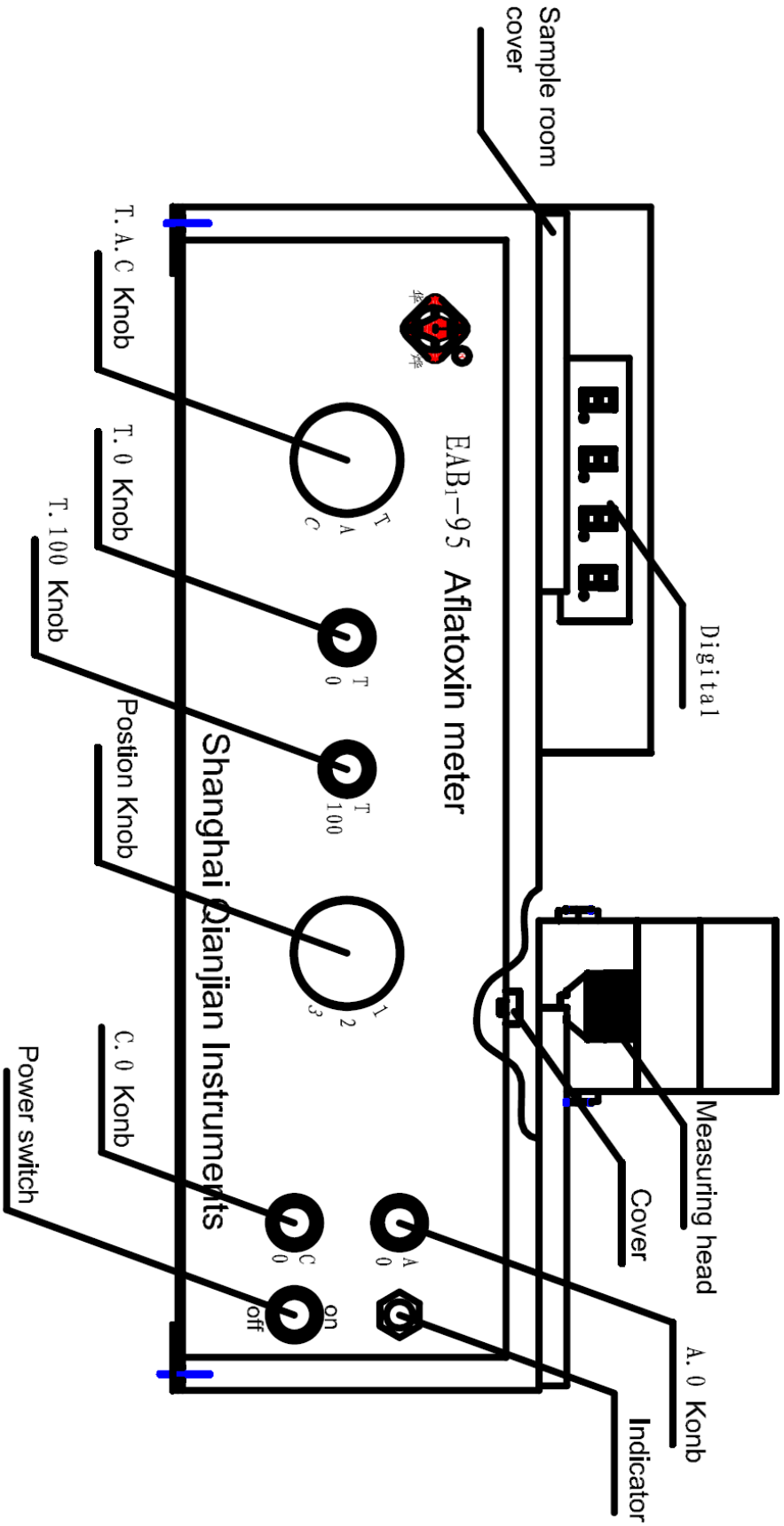
As —" A" value in sample well.

D — Sample dilution factor.

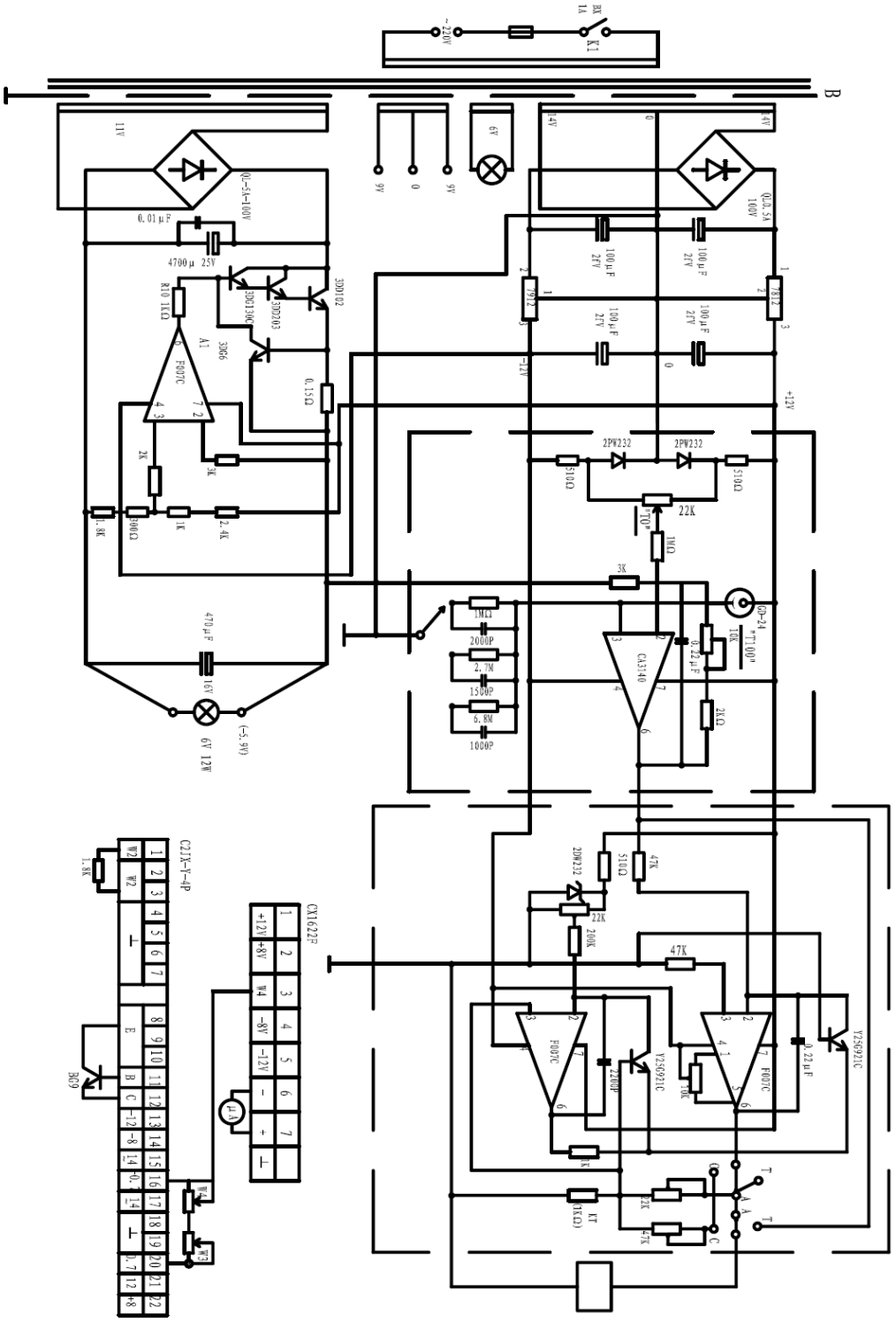
M — Sample weight, g.

V —The volume for sample extract, ml.

# Ⅴ Aflatoxin Microplate Reader Sketch



# Ⅵ The Principle Diagram for Electronics System



Electric for Principle Diagram

## Ⅶ The Test Kit Storage

7.1 Store the test kit at 2~8 ℃.Do not freeze.

7.2 Term of validity is in 12 months. If dilute C reagent, it is in 6 months.

## Ⅷ Toleration Standard in the Samples

(GB2671 — 81, GB8381 — 87)

| Sample | Toleration（μg/kg） |
|---|---|
| Infant milk product | -- |
| Other food, beans, fermenting food | ≤ 5 |
| Rice, edible oil | ≤ 10 |
| Food grade corn | ≤ 20 |
| Meat chicken, growing chicken match feed | ≤ 10 |
| Mixing or match feed | ≤ 20 |
| Cake of corn, peanut for feed | ≤ 50 |

## Ⅸ Notice

9.1 the B reagent and C reagent is poisonous, should avoid to pollute person and food.

Decontamination of glassware and $AFB_1$solution is best marinated in 5% NaClO ( sodium hypochlorite solution) overnight.

9.2 The reagent of the different batch number can't mix and use.

9.3 The instrument hull must be dependable to connect on earth.

9.4 The safekeeping of the instrument should notice protection against the dust and tide. Avoid contact of eroding air with it. When the instrument does not work, must draw the left cover of the sample and make light door close. Cover the dustproof cover.

9.5 the instrument should place on the environment of a weaker shine and stable work, avoiding work in light.

## Ⅹ Maintenance and Service

10.1 The customer should obey the instrumentation rules of the conveyance and safekeeping and operation, environment temperature is 5 ℃ ~35 ℃ , the opposite humidity is not over 85%.

10.2 Discover that instrument can’t work normally because of manufacturing quantity problem for a period of one year from the consignment date. The company will is the responsible for maintenance and return and exchange.

10.3 The kit is as waste product, the company can supply it for a long period.

## Accessory of the Instrument

| | |
|---|---|
| Aflatoxin Microplate Reader host | One |
| Three core wire with plug | One |
| Test kit | One |
| 0.5A Fuse | Five |
| Micropipet | One |
| Qualified certificate | One |

**Shanghai QianJian instruments Products**

| Nitrogen Analyzer | |
|---|---|
| Distillation Device | Digest Stove |
| **KDN–102C**<br>**KDN–103F**<br>**KDN–2008** | **HYP 1004 four bores**<br>**HYP-1008 eight bores**<br>**HYP-1014 fourteen bores**<br>**HYP-1020 twenty bores**<br>**HYP-1040 fortybores** |
| **SZC–C，SZC-D Fat Determinator** | |
| **SLQ–6 Crude fiber Determinator** | |
| **EAB1–2000 Aflatoxin meter (include printer)** | |
| **EAB1–95 Aflatoxin meter** | |

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