Fumonisin B1 ELISA kit

1. Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Fumonisin B1 in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Fumonisin B1 in the sample and the coupling antigens pre-coated on the micro-well stripes compete for anti-Fumonisin B1 antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the Fumonisin B1 concentration in the sample. This value is compared to the standard curve and the Fumonisin B1 concentration is subsequently obtained.

2. Technical specifications

Sensitivity : 10ppb

Detection limit:

Grain, corn, feed ..... 0.5 ppm

Linear range:

10ng/mL~200ng/mL

Recovery rate:

Grain, corn, feed ..... 95±25%

Cross-reaction rate:

Fumonisin B1 ..... 100%

Precision:

Intra-assay CV<10%;

Inter-assay CV<15%

3. Components

1） Micro-well strips:96wells(12 strips with 8 removable wells each)
2） 5× standard solution (0.5 mL each): 0 ppb,10 ppb, 50 ppb, 100 ppb,200 ppb
3） Anti-FB1 monoclonal antibody solution(6ml) ..... ready for use directly
4） Enzyme conjugate (12 mL) ..... ready for use directly
5） Substrate solution (12 mL) ..... ready for use directly
6） Stop solution (6 mL) ..... ready for use directly
7） 10× concentrated washing buffer (30 mL) ..... dilute for use

4. Materials required but not provided

1) **Equipments:** microplate reader, oscillator,grinder, balance(a reciprocal sensibility of 0.01 g), printer, centrifuge, measuring pipets,.
2) **Material** :Graduated cylinder, funnel, flask, rapid qualitative filter paper
3) **Micropipettors:** single-channel 20~200 µL, 100~1000 µL; and multi-channel 250 µL;
3) **Reagents:14%**Methanol, Sodium chloride (AR),deionized water

Skype:missyan819 **Tel:** 86-755-21567288 **Fax:** 86-755-28938800
Email/MSN:info@lsybt.com,lvshiyuan711@gmail.com **Website**: http://www.lsybt.com
Address: Rm.507, No.2, longgang Overseas Venture Park,Shenzhen,China. 518172

5. Sample treatment

Instructions (The following points must be dealt with before the pre-treatment)

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents;

2) Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Make 30ml 70% Methanol solution:such as

21ml Methanol +9ml deionized water (at volume= 7:3)

Sample treatment:

1)Take 5g smashed sample into centrifuge,add 1.25g Sodium chloride (AR),add 25ml,70% Methanol solution,shake severely for 3 min

2) Filter at rapid qualitative filter paper

3) Take 1ml filtrate,add 9ml 1% NaCl solution for diluting.

4) take 50 µL for analysis.

Fold of dilution of sample :50

6. ELISA procedures

Tips:

1)Be careful when using the standard solution,there are FB1 in them.

2)The time of adding samples each time should be finished in 5 mins.

1 Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.

2 Solution preparation:dilute 30 mL of the concentrated washing buffer (10×concentrated) with the distilled or deionized water to 300 mL (or just to the required volume) for use;

3 Take needed micro-wells into microplate,wash the microplate with the washing buffer at 250 µL/well for two times.,each time soak the well with the washing buffer for 3 min

4 Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.

5 Add 50 µL of the sample and standard solution to separate duplicate wells, then add 50 µL Anti-FB1 monoclonal antibody solution to,seal the microplate with the cover membrane,and incubate at 37 ℃for 90 min;

6 Abandon the liquid of the microplate,wash the microplate with the washing buffer at 250 µL/well for three times. Each time soak the well with the washing buffer for 3min,after the last time, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips);

7 Add 100µL/well of enzyme conjugate to each well, shake properly, seal the microplate with the cover membrane, and incubate at 37 ℃ for 60 min;

8 Abandon the liquid of the microplate,wash the microplate with the washing buffer at 250 µL/well for three times. Each time soak the well with the washing buffer for 3min,after the last time, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the

clean tips);

9 Coloration: add 100 µL of the substrate solution into each well. Mix gently by shaking the plate manually, and incubate at 37 ℃for 15 min in the dark for coloration;

10 Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well.

7. Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the content of Fumonisin B1.

7.1 Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the testing sample with that of the standard solution.

Quantitative determination

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the testing sample and the standard solution divided by the OD value (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average (double wells) OD value of the testing sample or the standard solution

B_0—the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Fumonisin B1 standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the testing sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Fumonisin B1 concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software)

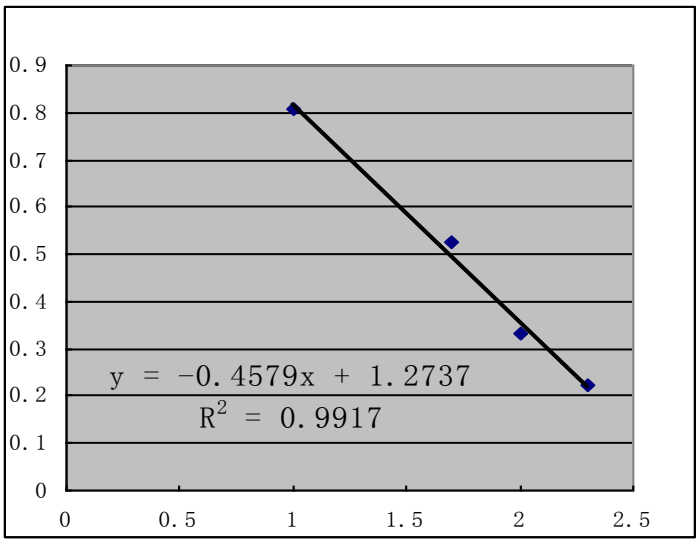

Chart (for reference only)

8. Precautions

1 Bring all reagents and micro-well strips to the room temperature (20-25℃).

2 Return all reagents to 2-8℃ immediately after use.

3 The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.

4 For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

5 The room temperature below 20℃ or the temperature of the reagents and the testing samples being not returned to the room temperature (20-25℃) will lead to a lower standard OD value.

6 Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.

7 Mix evenly, otherwise there will be the undesirable reproducibility.

8 The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.

9 Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.

10 Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.

11 Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of standard solution 1（0 ppb）of less than 0.5 indicates its degeneration.

12 Colouration time is about 20 min, if the color is light, prolong the time of colouration but don't exceed 30 min.

13 The optimum reaction temperature is 37 ℃, and too high or low temperatures will result in the changes in the detecting sensitivity and OD values.

Skype:missyan819 **Tel:** 86-755-21567288 **Fax:** 86-755-28938800
Email/MSN:info@lsybt.com,lvshiyuan711@gmail.com **Website**: http://www.lsybt.com
Address: Rm.507, No.2, longgang Overseas Venture Park,Shenzhen,China. 518172

9. Storage and expiry date

Storage: stored at 2-8 ℃, not frozen.

Expiry date: 12 months; date of production is on the box

Shenzhen Lvshiyuan Biotechnology Co.,Ltd makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made of standard quality. If any materials are defective, Lvshiyuan Biotechnology will provide a replacement product.There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Lvshiyuan Biotechnology shall not be liable for any damages, including special or consequential damage, or expense arising directly ot indirectly from the use of the this product.

Green Earth depends on everyone' efforts

"Build of green Earth needs the cooperation of you and me"

Skype:missyan819 **Tel:** 86-755-21567288 **Fax:** 86-755-28938800
Email/MSN:info@lsybt.com,lvshiyuan711@gmail.com **Website**: http://www.lsybt.com
Address: Rm.507, No.2, longgang Overseas Venture Park,Shenzhen,China. 518172