KIT FOR RAPID DETECTION OF MOLECULE INDICATING TOXIGENICITY OF AFLATOXIN-PRODUCING FUNGI, AND USE THEREOF

TECHNICAL FIELD

The present invention relates to a kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi, and use thereof.

BACKGROUND

Aflatoxin, with strong toxicity and great harm, is a contaminant that contaminates most food varieties, and has shown an increasing trend of contamination in recent years, thus seriously threatening food safety and people's health. Aflatoxin is one of the most toxic mycotoxins in nature, among which aflatoxin B1 is a class I carcinogen identified by the International Agency for Research on Cancer (IARC), which has caused many poisoning incidents in human and animal populations and become one of the main reasons for the high incidence of liver cancer. According to the statistics of data retrieved from Web of Science in the past five years, there are more than 110 varieties of aflatoxin contaminated foods and raw materials, ranking first among contaminants. However, there is still no research example of molecular early warning before contamination by microbial toxins such as aflatoxins at home and abroad, and it is difficult to meet the urgent needs of early warning.

The existing aflatoxin early warning methods are mainly established based on aflatoxin detection technology and is used for toxin contamination level evaluation or post-harvest contamination degree and consumption risk assessment. Once detected, contamination often has occurred, thus it is difficult to meet the urgent requirements of early warning and guidance for prevention and control. Using aflatoxin detection technology to carry out contamination early warning is the successful experience of food safety supervision in developed countries such as Europe and America. The EU Rapid Alert System for Food and Feed (RASFF) is to obtain the aflatoxin content in food and feed using the limit standard and testing for quick early warning of food and feed imported into the European Union from countries. Based on aflatoxin detection technology and contamination monitoring data, research institutions in the United

States have studied and established early warning models such as a multivariate logistic regression analysis model and a superimposed Gaussian processing model, which are mainly used to assess the degree of mycotoxin contamination and consumption risks of post-harvest corn and other agricultural products.

Based on the research progress in the past two decades at home and abroad, the fundamental reason lies in the lack of molecular detection technology for early warning of aflatoxin. In order to deal with this bottleneck problem, after more than ten years of research, the inventor team successfully discovered a molecule indicating the toxigenicity of the aflatoxin-producing fungi, and the early warning function of the molecule, which becomes the first molecule for early warning, and successfully invented a kit for rapid detection of the molecule indicating the toxigenicity of the aflatoxin-producing fungi on this basis, and the kit was used to identify the aflatoxin-producing ability of strains, namely, the toxigenicity, and to find out whether there is aflatoxin-producing strains of strong toxigenicity in farmland, agricultural products and feed, so as to provide a scientific basis for timely detecting aflatoxin contamination risk and early prevention and early control.

SUMMARY

Aiming at the defects of the prior art, the present invention provides a kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi, and use thereof, and the kit is used for identifying the aflatoxin-producing ability of the strains of genus *Aspergillus* and identifying whether there are aflatoxin-producing strains with strong toxigenicity in products such as farmland, agricultural products, traditional Chinese medicinal materials and feed, and is easy to popularize and apply.

In order to solve the above-mentioned technical problems, the technical solution adopted by the present invention is as follows:

provided is a kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi, comprising an ELISA plate coated with nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi at the bottoms of wells, polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing

fungi, a pure solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi functioning as a reference material, a Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus*, horseradish peroxidase-labeled antibodies that bind to the polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, a chromogenic solution for ELISA, a stop solution for ELISA, and a sample diluent;

the molecule indicating the toxigenicity of the aflatoxin-producing fungi refers to an AFT-YJFZP008 peptide, of which the amino acid sequence is shown as in SEQ ID NO. 1.

According to the above solution, the ELISA plate coated with nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi at the bottoms of wells is prepared by the following method: dissolving the nanobodies or monoclonal antibodies against AFT-YJFZP008 in ELISA coating buffer to form a coating solution of 0.2-8.0 μg/mL, then adding the coating solution into the ELISA plate (in an amount of 100-200 μL per well), after leaving the coating solution at 4°C overnight or at 37°C for no less than 2 hours, removing the coating solution in the ELISA plate, then washing the ELISA plate with a conventional washing solution for ELISA; then adding a conventional blocking solution for ELISA (in an amount of 200-300 μL per well), after leaving the blocking solution at room temperature or at 37°C for blocking for no less than 1 hour, discarding the blocking solution, and then washing the ELISA plate with the conventional washing solution for ELISA.

The ELISA coating buffer refers to a conventional carbonate buffer, and a preparation method of the conventional carbonate buffer comprises: weighing 1.465 g of $NaHCO_3$ and 0.795 g of Na_2CO_3, and adding deionized water to make up to a volume of 500 mL.

In the above-mentioned solution, the polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi are rabbit-derived polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, and the horseradish peroxidase-labeled antibodies are horseradish peroxidase-labeled goat anti-rabbit antibodies.

In the above solution, the polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi and the nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi are of different animal origin, and specifically, the molecule indicating the toxigenicity of the aflatoxin-producing fungi obtained through research can be directly used as an antigen to prepare and obtain the nanobodies or monoclonal antibodies and the rabbit-derived polyclonal antibodies;
the nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi can be prepared and obtained by using the AFT-YJFZP008 as an immune antigen, immunizing alpaca or Balb/c mice in a conventional way, and then using the known conventional nanobody or mouse-derived monoclonal antibody preparation technical scheme.

The rabbit-derived polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi can be prepared and obtained by using the AFT-YJFZP008 as an immunization antigen, immunizing experimental rabbits such as New Zealand white rabbits in a conventional manner, and then using the known conventional rabbit-derived polyclonal antibody preparation technical scheme.

The Czapek-Dox medium is a conventional Czapek-Dox medium, and can be prepared by oneself or directly obtained from commercial products.

The horseradish peroxidase-labeled goat anti-rabbit antibodies can be directly obtained from commercial products.

According to the above solution, the chromogenic solution for ELISA refers to a conventional hydrogen peroxide and TMB chromogenic solution for ELISA.

The stop solution refers to a conventional color development stop solution for ELISA: a 2 mol/L sulfuric acid aqueous solution, and a preparation method of the sulfuric acid aqueous solution comprises: adding 44 mL of concentrated sulfuric acid into 300 mL

of deionized water, carrying out stirring until the mixture is cooled, and finally making up to a volume of 400 mL.

The sample diluent refers to 0.01 mol/L phosphate buffer containing 0.1% sorbitol and soft white sugar, and a preparation method of the phosphate buffer comprises: adding 0.5 g of sorbitol and 0.5 g of sugar, 4.0 g of NaCl, 1.45 g of $Na_2HPO_4 \cdot 12H_2O$, 0.1 g of KCl and 0.1 g of KH_2PO_4 into deionized water to make up to a volume of 500 mL.

The present invention further provides application of the kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi to the identification of the aflatoxin-producing ability of the strains of genus *Aspergillus* or the identification of whether there are aflatoxin-producing strains with strong toxigenicity in farmland, agricultural products and feed.

Application method:
culturing a strain to be identified or a sample to be identified in the Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus* in the kit, and preparing a solution to be tested of the strain to be identified or the sample to be identified;
diluting the solution to be tested of the strain to be identified or the sample to be identified with the sample diluent, adding the diluted solution to be tested of the strain to be identified or sample to be identified into the wells of the ELISA plate in the kit in an amount of 100-200 μL per well, after leaving the diluted solution at room temperature or at 37°C for reacting for no less than 1 hour, discarding the post-reaction liquid, and washing the ELISA plate;
then adding the polyclonal antibodies against AFT-YJFZP008 in the kit into the wells of the ELISA plate in an amount of 100-200 μL per well, after leaving the polyclonal antibodies at room temperature or at 37°C for reacting for no less than 1 hour, discarding the liquid, and then washing the ELISA plate;
then adding the horseradish peroxidase-labeled antibodies in the kit into the wells of the ELISA plate in an amount of 100-200 μL per well, after leaving the horseradish peroxidase-labeled antibodies at room temperature or at 37°C for reacting for no less than 1 hour, discarding the liquid, and then washing the ELISA plate; and

then adding the chromogenic solution for ELISA and the stop solution for ELISA in the kit successively, and finally reading and calculating the concentration of AFT-YJFZP008 in the sample to be tested by a microplate reader.

According to the above-mentioned solution, the method further comprises: evaluating the detection results: obtaining the content of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the solution to be tested of the strain to be identified in unit volume according to results displayed by the microplate reader, and analyzing the aflatoxin-producing ability of the strain to be identified, wherein the higher the concentration of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing ability in the strain to be identified, the stronger the aflatoxin-producing ability, that is, the toxigenicity of the strain to be identified; and
obtaining the content of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the sample solution to be identified in unit volume according to results displayed by the microplate reader, and judging whether the sample to be identified contains an aflatoxin-producing strain with strong toxigenicity, wherein if the concentration of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the sample to be identified is high, it is indicated that the sample to be identified contains an aflatoxin-producing strain with strong toxigenicity.

According to the above solution, the solution to be tested was replaced with the pure solution, of a series of concentrations, of the molecule AFT-YJFZP008 (100-200 μL per well) indicating the toxigenicity of the aflatoxin-producing fungi functioning as a reference material for making a standard curve for calculating the concentration of AFT-YJFZP008 in the sample to be tested.

According to the solution, the preparation of the solution to be tested of the strain to be identified is as follows: culturing the strain to be identified to obtain the solution to be tested of the strain to be identified, specifically, culturing the strain to be identified in the conventional Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus* at an ambient temperature of 15-35°C for no less than 12 hours, then fully homogenizing a mixture of the culture medium and the culture, and then diluting the mixture 1-10 times with sterile water to obtain the solution to be tested of the strain to be identified;

the preparation of the sample solution to be identified comprises: culturing the sample to be identified and carrying out dilution to obtain a solution to be tested of the sample to be identified;

specifically, weighing the sample to be identified, transferring the sample to be identified into the sample diluent, shake the obtained mixture at room temperature until the mixture is uniform to prepare a uniform dispersion of the sample to be tested, adding 10-1000 μL of the uniform dispersion of the sample into a 6-600 mL conventional Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus* for shaking culture at 15-35°C and at 200±50 rpm for 6-24 hours, and then carrying out sampling to form the solution to be tested of the sample to be identified.

According to the above solution, the samples are farmland, agricultural products, traditional Chinese medicinal materials, feed and other products.

The kit can be used for identifying the aflatoxin-producing ability of the strain of genus *Aspergillus*, and the higher the content of the molecule indicating the toxigenicity of the aflatoxin-producing fungi in the strain to be identified, the stronger the aflatoxin-producing ability of the strain to be identified; the kit can be used for identifying whether there are aflatoxin-producing strains with strong toxigenicity in farmland, agricultural products, traditional Chinese medicinal materials, feed and other products, and if the content of the molecule indicating the toxigenicity of the aflatoxin-producing fungi in the sample to be identified is high, it is indicated that the sample to be identified contains aflatoxin-producing strains with strong toxigenicity;

the toxigenicity of the aflatoxin-producing strain is an index for measuring the aflatoxin producing ability of the aflatoxin-producing strain, and the stronger the toxigenicity of the aflatoxin-producing strain, the greater the amount of aflatoxin produced by the aflatoxin-producing strain under the same time and culture conditions. The present invention utilizes the molecule indicating the toxigenicity of the aflatoxin-producing fungi obtained through research, combining with the nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, as well as the rabbit-derived polyclonal antibodies, and establishes a sandwich immune detection method by these antibodies and finally assembles the parts into a kit; in the application of the kit, by using the characteristics

that the measured concentration of the molecule indicating the toxigenicity of the aflatoxin-producing fungi is positively correlated with the toxigenicity of the strain, the kit can be used for the identification of the aflatoxin-producing ability of the strains of genus *Aspergillus* and the identification of whether there are aflatoxin-producing strains with strong toxigenicity in farmland, agricultural products and feed, is practical and easy to popularize and apply, provides a key means and scientific basis for timely detecting aflatoxin contamination risk and early prevention and early control, and are of great significance to promoting high-quality development of the agricultural industry and ensuring food safety.

The beneficial effects of the present invention are:
1, the kit for rapid detection of the molecule indicating the toxigenicity of the aflatoxin-producing fungi is provided, and can be used for rapidly and quantitatively detecting the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi; 2, the kit can be used for identifying and comparing the aflatoxin-producing ability of aflatoxin-producing strains of the genus *Aspergillus*; 3, the kit can be used for identifying whether there are aflatoxin-producing strains with strong toxigenicity in farmland, agricultural products and feed; 4, the kit is easy to operate, practical and easy to popularize and apply; and 5, the kit is of great significance to promoting high-quality development of the agricultural industry and ensuring food safety.

DETAILED DESCRIPTION

Example 1 Preparation of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi capable of functioning as a reference material in the kit

Czapek-Dox medium was prepared according to the following formula: 3% (w/v) sucrose, 0.3% (w/v) $NaNO_3$, 0.1% (w/v) K_2HPO_4, 0.05% (w/v) $MgSO_4 \cdot 7H_2O$, 0.05% (w/v) KCl, 0.001% (w/v) $FeSO_4$, at pH6.5 to obtain the Czapek-Dox medium. A total of 10 aflatoxin-producing strains published by the literature of open access “Study on the Distribution, Toxigenicity and Infection of *Aspergillus flavus* in Typical Peanut Producing Areas in China” (Thesis for Master’s Degree of the Chinese Academy of Agricultural Sciences, author: Zhang Xing, Page 33) and including HLJ-1, HeNZY-2, HuBha-24, JXZS-29-2, LNct-6, GXfc-34, GDZJ-122-2, JSnt-1, HuNdx-7 and HBHA-8-17 were randomly selected and inoculated into the above Czapek-Dox medium

separately for culturing at 28°C and 200 rpm for 5 days, the strain culture was fully homogenized by conventional methods for disrupting the cells, and then the obtained liquid was purified by using the conventional protein purification system method, protein electrophoresis method, immunoaffinity method and other methods to obtain the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi. The experimental results showed that AFT-YJFZP008 could be prepared from the cultures of all the aflatoxin-producing strains. Under the same culture conditions, the amount of AFT-YJFZP008 prepared from HBHA-8-17 was the largest, while the amount of AFT-YJFZP008 prepared from HLJ-1 was the least.

The immunoaffinity method refers to diluting the cell disruption liquid of an aflatoxin-producing strain with a loading liquid, filtering the diluted cell disruption liquid through a conventional filter paper, and then adding the filtered cell disruption liquid into an immunoaffinity column continuously, when the liquid basically runs out, washing the immunoaffinity column with a conventional leacheate, finally eluting the immunoaffinity column with glycine buffer of pH 2.2 or a 70% methanol aqueous solution, after collecting the eluent, removing the solution in time by a conventional ultrafiltration centrifugation method, and then dissolving the protein left in the ultrafiltration centrifuge tube from the ultrafiltration centrifuge tube by sterile water to obtain the aqueous solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi.

The initial acquisition of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi was excavated by:

the method for excavating the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi is as follows:

(1) taking an *Aspergillus flavus* strain with strong toxigenicity, and culturing to obtain a strain culture and an extracellular secretory protein mixture; then disrupting the cells in the strain culture to obtain an intracellular protein mixture; combining the extracellular secretory protein mixture and the intracellular protein mixture, and adding carbodiimide for coupling to obtain the aflatoxin antigen;

(2) immunizing the test animal with the aflatoxin antigen to obtain a nanobody library or a monoclonal antibody library;

(3) obtaining a protein combined solution for *Aspergillus flavus* strains with different toxigenicity, and detecting the protein combined solution for *Aspergillus flavus* strains with different toxigenicity by using the antibodies in the antibody library obtained in step (2) to obtain a series of detection signals; and

(4) finding out nanobody with the detection signal positively correlated with the toxigenicity of the *Aspergillus flavus* strains, namely the antibody against the molecule indicating the toxigenicity of the *Aspergillus flavus* strains, wherein the protein corresponding to the antibody against the molecule indicating the toxigenicity of the *Aspergillus flavus* strains was the excavated molecule indicating the toxigenicity of the *Aspergillus flavus* strains.

In the above solution, the *Aspergillus flavus* strain with strong toxigenicity in step (1) was obtained by isolation and identification from nature by a conventional method, or obtained by artificial transformation, and the toxigenicity thereof was identified by NY/T 2311-2013 standard method to be no less than 10 μg/kg.

There were at least 3 *Aspergillus flavus* strains with different toxigenicity described in step (3), and the toxigenicity thereof was identified by NY/T 2311-2013 standard method to show at least three levels: high, medium and low.

The culture medium adopted in the culturing of the *Aspergillus flavus* strain with strong toxigenicity is Czapek-Dox medium or other nutrients that can be used for normal growth of *Aspergillus flavus*, the culturing time is not less than 12 hours, and the ambient temperature for culturing is 15 to 35°C.

The disruption of the cells in the strain culture was carried out by a conventional liquid nitrogen grinding method or a cell disruption instrument and the other methods.

The carbodiimide is used in an amount of 0.005 to 0.1 g added into per 1.0 mL of the combined extracellular secretory protein mixture and intracellular protein mixture.

The coupling reaction refers to reacting at 15-37°C for 2-6 hours and reacting at 4-10°C overnight.

The immunization is carried out in a conventional manner, and the aflatoxin antigen is inoculated. The test animals refer to mice or alpacas or other test animals with similar effects.

According to the above-mentioned solution, the antibody preparation process refers to a conventional nanobody preparation technical process or a conventional cell fusion-based hybridoma monoclonal antibody preparation technical process.

According to the above-mentioned solution, the detection of protein of the *Aspergillus flavus* strains with different toxigenicity adopted a conventional Western Blot technical process, namely, the proteins of the *Aspergillus flavus* strains with different toxigenicity were transferred to a nitrocellulose membrane, and then the antibodies in the antibody library were utilized to detect the proteins by a direct method or an indirect method, or by using the other technical processes with similar efficacy.

According to the above-mentioned solution, the direct method refers to coupling the antibodies in the antibody library to the signal material by conventional methods, and then enabling the antibodies to be in immune binding reaction with the corresponding proteins transferred to the nitrocellulose membrane.

According to the above-mentioned solution, the indirect method refers to enabling the antibodies in the antibody library to firstly be in immune binding reaction with the corresponding proteins transferred to the nitrocellulose membrane, and then enabling the conjugate of the second antibody and the signal material to be in immune binding reaction with the antibodies bound to the nitrocellulose membrane.

The signal material in the above detection is horseradish peroxidase or colloidal gold or fluorescent material or other materials with similar efficacy. The detection signal is a signal for color reaction or a spot signal or a fluorescent signal.

Example 2 Preparation of the nanobodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

The nanobodies can be prepared by using AFT-YJFZP008 as an immune antigen, immunizing the alpaca or the Balb/c mouse in a conventional way, and then using the known conventional nanobody or mouse-derived monoclonal antibody preparation technical scheme.

The AFT-YJFZP008 prepared above was dissolved in conventional PBS or normal saline until the concentration was not less than 0.1 mg/mL, then mixed with Freund's complete adjuvant in an equal volume for emulsification, and alpaca was immunized by subcutaneous injection or intradermal multi-site injection on the back, followed by immunization enhancement once every 2-4 weeks, and during immunization enhancement, Freund's complete adjuvant was replaced by Freund's incomplete adjuvant. The immune effect was monitored by adopting a routine ELISA procedure until the serum titer of the alpaca no longer increased, the following operations were carried out according to the methods described in the patent document CN103866401A, such as collection of the venous blood of the immunized alpaca, extraction of the total RNA, cDNA synthesis, amplification of VHH gene, recovery of VHH gene fragment, ligation of VHH gene with pCANTAB 5 E(his) vector treated by double enzyme digestion, electrotransformation of ligation products, construction of gene bank of nanobodies and rescue of gene bank of nanobodies, etc., and finally the rescued gene bank of nanobodies was obtained.

The AFT-YJFZP008 prepared above was immobilized on a solid-phase carrier such as a 96-well ELISA plate according to the gradient of 8 μg/well, 2 μg/well, 0.5 μg/well, and 0.1 μg/well, and the rescued gene bank of nanobodies is subjected to panning for 2 to 4 times according to the method described in the patent document CN103866401A, and identifying the antibody produced by each phage clone by AFT-YJFZP008 and indirect non-competitive ELISA, wherein the phage corresponding to the positive result is a positive phage clone, and then preparing the nanobodies by a conventional method for preparing the nanobodies by using the positive clone, namely the nanobodies against AFT-YJFZP008, for further application research work, and the nanobodies with strong specificity and high affinity characterized by an ELISA method were preferred.

Example 3 Preparation of the monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

The monoclonal antibodies can be prepared by using AFT-YJFZP008 as an immune antigen, immunizing the alpaca or the Balb/c mouse in a conventional way, and then using the known conventional nanobody or mouse-derived monoclonal antibody preparation technical scheme.

The AFT-YJFZP008 prepared above was dissolved in conventional PBS or normal saline until the concentration was not less than 0.1 mg/mL, then mixed with Freund's complete adjuvant in an equal volume for emulsification, and BALB/c mice were immunized by subcutaneous injection or intradermal multi-site injection on the back, followed by immunization enhancement once every 2-4 weeks, and during immunization enhancement, Freund's complete adjuvant was replaced by Freund's incomplete adjuvant. The immune effect was monitored by adopting a routine ELISA procedure until the serum titer of the BALB/c mice no longer increased, then the operations were completed according to the methods described in the patent document CN103866401A: isolation of the spleen cells of the immunized mice, fusion of the spleen cells and the mouse-derived myeloma cells SP2/0, and the selective culture of the hybridoma cells by a semi-solid medium, and after the needle-like white spots grow on the semi-solid medium, the white spots were respectively picked into a 96-well culture plate with a built-in conventional hybridoma culture medium, so that the monoclonal hybridoma resource bank was obtained.

The culture supernatant of the monoclonal hybridoma, namely the monoclonal antibody was obtained by referring to the method of patent document CN103849604A, the AFT-YJFZP008 prepared above was immobilized on a solid-phase carrier such as a 96-well ELISA plate according to the gradient of 8 μg/well, 2 μg/well, 0.5 μg/well, and 0.1 μg/well, and then each monoclonal antibody was identified by an indirect non-competitive ELISA procedure, positive clones were selected to obtain monoclonal antibodies against AFT-YJFZP008 and were used for further application research. The monoclonal antibody against AFT-YJFZP008 detected with strong specificity and high affinity was preferred.

Example 4 Preparation of rabbit-derived polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

The rabbit-derived polyclonal antibodies can be obtained by using AFT-YJFZP008 as an immune antigen, and immunizing the New Zealand white rabbits and other test rabbits in a conventional way, and then using the known conventional rabbit-derived monoclonal antibody preparation technical scheme.

The AFT-YJFZP008 prepared above was directly used as an antigen, and was mixed with Freund's complete adjuvant in an equal volume in the form of a solution with a concentration of no less than 0.1mg/mL for emulsification, and New Zealand white rabbits were immunized by subcutaneous injection or intradermal multi-site injection on the back, followed by immunization enhancement once every 2-4 weeks, and during immunization enhancement, Freund's complete adjuvant was replaced by Freund's incomplete adjuvant. The immune effect was monitored by adopting a routine ELISA procedure until the serum titer of the immunized animals no longer increased, the serum of the immunized animal, namely the rabbit-derived polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, was prepared by a conventional method.

Example 5 Establishment of a rapid immunodetection method for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

The basic operation procedure of a rapid immunodetection method for AFT-YJFZP008, namely, the double-antibody sandwich indirect non-competitive ELISA method, was as follows: the ELISA plate was coated with the nanobodies against AFT-YJFZP008 and was washed; the blocking solution was added for blocking and the ELISA plate was washed; AFT-YJFZP008 or the solution to be tested was added for reaction, and the ELISA plate was washed; the rabbit-derived polyclonal antibodies against AFT-YJFZP008 were added for reaction and the ELISA plate was washed; the horseradish peroxidase-labeled goat anti-rabbit antibodies were added for reaction, and the ELISA plate was washed; a chromogenic solution was added for reaction; the stop solution was added, and the results were read and calculated by a microplate reader. The following work is done using this basic procedure.

Determination of antibody concentration: multiple parallel experiments were carried out at the same time by the designed checkerboard titration method, and different concentrations of the nanobodies were used for coating, additionally, the rabbit-derived polyclonal antibodies were set to have different concentrations, and finally appropriate working concentrations for the nanobodies and the rabbit-derived polyclonal antibodies were determined according to the results, namely, under the principle of reducing the amount of antibodies, the two antibody concentrations corresponding to a point with a value at OD450nm approximate to 1.0 are selected, ELISA results showed that, the detection can also be achieved by using other concentrations of the nanobodies and the rabbit-derived polyclonal antibodies, and a suitable coating concentration of the nanobodies was 2.2 μg/mL, and a suitable coating concentration of the rabbit-derived polyclonal antibodies was 2.8 μg/mL.

Determination of optimal antibody coating conditions: coating is the first step in the study of the ELISA method, and the coating effect has a very critical impact on the results of ELISA. To determine the effect of different coating conditions on the detection results, three different conditions were selected to coat the wells and include coating at 4°C overnight, coating at constant temperature of 37°C for 2 hours and coating at constant temperature of 37°C for 1 hour. The detection results showed that all the three coating methods studied could achieve the coating, and the optimal coating condition was coating at 4°C overnight.

Determination of optimal blocking agent: after coating of the antibodies was completed, in order to avoid the interference of the unoccupied sites in the ELISA plate wells with the subsequent steps of ELISA, some inert proteins, i.e., blocking agents, need to be used to occupy these sites, and inappropriate blocking agents will non-specifically bind to the second antibody, resulting in a false-positive situation. In this study, three different blocking agents, 3% BSA /PBST, 3% skimmed milk powder /PBST and 5% skimmed milk powder /PBST were used for blocking. The research results showed that although the three blocking agents could achieve the purpose of blocking to different degrees, the blocking effect of 5% skimmed milk powder/PBST was the best, so that the 5% skimmed milk powder/PBST was the best blocking agent.

Determination of the blocking time: in this study, three different blocking times of 1 hour, 2 hours and 3 hours at the constant temperature of 37°C were set for blocking. The test results showed that the value at OD450nm of positive well/the value at OD450nm of negative well was the largest under the setting of blocking for 2 hours at 37°C, and the value at OD450nm of positive sample >1, so 2 hours at the constant temperature of 37°C was the best blocking time.

Determination of the reaction time of the rabbit-derived polyclonal antibodies: three reaction times of 30 min, 50 min and 1 hour at the constant temperature of 37°C were adopted in the study. The test results showed that the optimal reaction time of the rabbit-derived polyclonal antibodies was 50 min at the constant temperature of 37°C.

Drawing of an ELISA standard curve of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi: the molecule AFT-YJFZP008 was diluted to 0.00003 ng/mL, 0.0003 ng/mL, 0.003 ng/mL, 0.03 ng/mL, 0.3 ng/mL, 3 ng/mL, 30 ng/mL and 300 ng/mL, respectively, and the diluent was added in an amount of 200 μL per well. The ELISA standard curve of AFT-YJFZP008 was drawn under the above optimal conditions. The correlation coefficient of the double-antibody sandwich ELISA method established under the above optimal conditions was 0.99, and the detection limit for the molecule AFT-YJFZP008 can reach 0.05 ng/mL, indicating that the detection method has good detection sensitivity and accuracy.

Evaluation on the specificity of the method: in order to evaluate the specificity of the above immunodetection method for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, several strains of fungi with certain homology with *Aspergillus flavus*, such as *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus ochraceus*, and *Fusarium moniliforme*, etc., were selected for research, and the cell disruption liquid of these fungal cultures was detected. The results are shown in Table 1. The above method showed no obvious cross-reaction with the protein of fungi sharing homology with *Aspergillus flavus*, indicating that the established immunodetection method for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi had good specificity.

Table 1 Measuring results of the specificity of the rapid immunodetection method for the molecule indicating the toxigenicity of the aflatoxin-producing fungi

Sample name	*Fusarium oxysporum*	*Aspergillus niger*	*Aspergillus ochraceus*	*Fusarium moniliforme*	AFT-YJFZP008-producing *Aspergillus flavus*	Negative
OD_{450nm} value	0.168	0.186	0.176	0.192	0.922	0.183

Evaluation on the repeatability of the method: in order to evaluate the repeatability of the above established immunodetection method for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, four aflatoxin-producing strains—positive 1, positive 2, positive 3, positive 4, and one non- aflatoxin-producing strain—negative 5 were selected randomly for the study, and the intra-plate and inter-plate data variation of the measuring results was analyzed. The results of the above study are shown in Table 2. The intra-plate coefficient of variation was calculated to be 0.6%-5.7%, and the inter-plate coefficient of variation was calculated to be 1.2%-9.1%, both below 10%. Therefore, the above method has good repeatability.

Table 2 Measuring results of the repeatability of the rapid immunodetection method for the molecule AFT-YJFZP008 indicating the toxigenicity

Number	Intra-plate mean value	Intra-plate coefficient of variation %	Inter-plate mean value	Inter-plate coefficient of variation %
Positive 1	1.034	0.9	1.033	1.3
Positive 2	1.692	0.6	1.675	1.2
Positive 3	0.593	3.3	0.587	6.5
Positive 4	0.660	5.7	0.663	9.1
Negative 5	0.184	4.1	0.175	7.2

Evaluation on the accuracy of the method: in order to evaluate the detection accuracy of the above method and investigate the practicability of the method at the same time, peanut and corn samples were selected as examples for research and evaluation. AFT-YJFZP008, a molecule indicating the toxigenicity of the aflatoxin-producing fungi, was added into the corn and peanut samples. The closer the recovery rate of the detection result was to 100%, the more accurate and practical the method was. The results of this study are shown in Table 3. The results showed that when the detection method established above was used to detect the molecule AFT-YJFZP008 indicating the toxigenicity in peanuts and corns, and the recovery rate reached 83%-106%, indicating that the method had high accuracy and could be applied to the detection of actual samples with good practicability.

Table 3 Additive recovery test results of the rapid immunodetection method for the molecule indicating the toxigenicity of the aflatoxin-producing fungi

Sample type	Addition amount (ng/mL)	Test value+standard deviation (ng/mL)	Mean recovery rate
within the test (n=3)			
	50	54.73±0.82	83%
peanut	10	8.65±1.23	85%
	2	2.14±0.29	87%
	50	51.92±2.53	98%
corn	10	8.75±0.39	95%
	2	1.93±0.24	96%
between the tests (n=5)			
	50	107.80±4.03	97%
peanut	10	8.25±0.89	106%
	2	2.08±0.19	102%
	50	106.21±3.25	102%
corn	10	9.45±1.13	94%
	2	1.97±0.19	96%

Example 6 Composition and shelf-life of a rapid immunodetection kit for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

The rapid immunodetection kit for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi is mainly composed of an ELISA plate coated with nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi at the bottoms of wells, rabbit-derived polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, a pure solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi functioning as a reference material, a Czapek-Dox medium, horseradish peroxidase-labeled goat anti-rabbit antibodies, a chromogenic solution for ELISA, a stop solution for ELISA, and a sample diluent.

The preparation method of the above ELISA plate coated with nanobodies or monoclonal antibodies against the molecule indicating the toxigenicity of the aflatoxin-producing fungi at the bottoms of the wells includes: dissolving the above nanobodies or monoclonal antibodies against AFT-YJFZP008 in ELISA coating buffer, a carbonate solution to form a coating solution of 2 μg/mL, then adding the coating solution to the 96-well ELISA plate with 100-200 μL per well, after leaving the coating solution at 4°C overnight or at 37°C for no less than 2 hours, removing the coating solution in the ELISA plate, then washing the ELISA plate with the conventional washing solution for ELISA; then adding the conventional blocking solution for ELISA in an amount of 200-300 μL per well, after leaving the blocking solution at room temperature or at 37°C for blocking for no less than 1 hour, discarding the blocking solution, washing the ELISA plate with the conventional washing solution for ELISA again, thus obtaining the ELISA plate coated with nanobodies or monoclonal antibodies against the molecule indicating the toxigenicity of the aflatoxin-producing fungi at the bottoms of the wells.

The nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi as described above can be prepared by the methods of Example 2 and Example 3.

The ELISA coating buffer refers to a conventional carbonate buffer, and a preparation method of the ELISA coating buffer is as follows: weighing 1.465 g of $NaHCO_3$ and 0.795 g of Na_2CO_3, and adding deionized water to make up to a volume of 500 mL.

The rabbit-derived polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi can be prepared by the method of Example 4.

The Czapek-Dox medium is a conventional Czapek-Dox medium, and can be prepared by oneself or directly obtained from commercial products.

The horseradish peroxidase-labeled goat anti-rabbit antibodies can be directly obtained from commercial products.

The chromogenic solution for ELISA refers to a conventional hydrogen peroxide and TMB chromogenic solution for ELISA.

The stop solution refers to a conventional color development stop solution for ELISA, which is a 2 mol/L sulfuric acid aqueous solution, and a preparation method of the sulfuric acid aqueous solution comprises: adding 44 mL of concentrated sulfuric acid into 300 mL of deionized water, carrying out stirring until the mixture is cooled, and finally making up to a volume of 400 mL.

The sample diluent refers to 0.01 mol/L phosphate buffer containing 0.1% sorbitol and sugar, and a preparation method of the phosphate buffer comprises: adding 0.5 g of sorbitol and 0.5 g of sugar, 4.0 g of NaCl, 1.45 g of $Na_2HPO_4 \cdot 12H_2O$, 0.1 g of KCl and 0.1 g of KH_2PO_4 into deionized water to make up to a volume of 500 mL.

The shelf-life study results of the above kits showed that the kits can be stored at 4°C for at least 6 months.

Example 7 Application of the rapid immunodetection kit for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi to identification and comparison of the toxigenicity of aflatoxin-producing strains

Step 1, preparation of solutions to be tested of strains to be identified: a total of 10 aflatoxin-producing strains published by the literature of open access “Study on the Distribution, Toxigenicity and Infection of *Aspergillus flavus* in Typical Peanut Producing Areas in China” (Thesis for Master’s Degree of the Chinese Academy of Agricultural Sciences, author: Zhang Xing, Page 33) and including HBZHX-21, HBXY-36, HBHA-1-4, GDZJ-6, HeNZY-2, HuBha-24, JSnt-1, HuNdx-7, GDZJ-108-19 and HBHA-8-17 were selected according to the toxigenicity of the strains, the strains to be identified were separately cultured in the Czapek-Dox medium in the kit at an ambient temperature of 15-35 °C for no less than 12 hours, the mixture of the culture medium and the culture was fully homogenized, and then diluted 1-10 times with the sample diluent in the kit to obtain a solution to be tested of the strain to be identified.

Step 2, measurement of the solution to be tested of the strain to be identified: the solution to be tested was added into the wells of the ELISA plate in the kit in an amount of 100-200 μL per well, or the solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi of a series of concentrations in the kit was added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the solution was left at room temperature or at 37°C for reaction for no less than 1 hour, the post-reaction liquid was discarded, the ELISA plate was washed, and then the rabbit-derived polyclonal antibodies against AFT-YJFZP008 in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the rabbit-derived polyclonal antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the horseradish peroxidase-labeled goat anti-rabbit antibodies in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the horseradish peroxidase-labeled goat anti-rabbit antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the chromogenic solution for ELISA and the stop solution for ELISA in the kit were sequentially added, and finally the concentration of AFT-YJFZP008 in a sample to be tested was read and calculated by a microplate reader.

According to the results of the above literature, the toxigenicity of the 10 strains including HBZHX-21, HBXY-36, HBHA-1-4, GDZJ-6, HeNZY-2, HuBha-24, JSnt-1,

HuNdx-7, GDZJ-108-19 and HBHA-8-17 was 0 μg/L, 0 μg/L, 3.8 μg/L, 4.9 μg/L, 67.2 μg/L, 81.7 μg/L, 192.0 μg/L, 204.4 μg/L, 297.4 μg/L and 1027.5 μg/L, respectively, indicating that HBZHX-21 and HBXY-36 were non-aflatoxin-producing strains, HBHA-1-4 and GDZJ-6 and the like were strains with weak toxigenicity, and HBHA-8-17 and GDZJ-108-19 and the like were strains with strong toxigenicity.

Step 3, evaluation on the identification results: the higher content of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the strain to be identified, the stronger the aflatoxin-producing ability, that is, the toxigenicity of the strain to be identified. According to the kit and the application technical scheme thereof, the measurement results of the concentrations of AFT-YJFZP008 of the 10 strains including HBZHX-21, HBXY-36, HBHA-1-4, GDZJ-6, HeNZY-2, HuBha-24, JSnt-1, HuNdx-7, GDZJ-108-19 and HBHA-8-17 were as follows: 0 ng, 0 ng, 7.8 ng, 11.2 ng, 43.7 ng, 51.5 ng, 99.4 ng, 109.0 ng, 166.5 ng and 562.3 ng in sequence. The results also showed that HBZHX-21 and HBXY-36 were non-aflatoxin-producing strains, HBHA-1-4 and GDZJ-6 and the like were strains with weak toxigenicity, and HBHA-8-17 and GDZJ-108-19 and the like were strains with strong toxigenicity. The measurement result is consistent with the strength order of toxigenicity of the strains disclosed in the literature of open access “Study on the Distribution, Toxigenicity and Infection of *Aspergillus flavus* in Typical Peanut Producing Areas in China”, and the identification results of the strains with strong toxigenicity and the strains with weak toxigenicity are consistent with those of the literature, demonstrating that the kit and the application technical scheme thereof provided by the present invention can be used for identifying the toxigenicity of the aflatoxin-producing strain, and the kit is simple in method and convenient, easy to operate and practical.

Example 8 Identification of whether there are aflatoxin-producing strains with strong toxigenicity in farmland using the rapid immunodetection kit for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

Step 1, preparation of solutions to be tested of samples to be identified: a total of four rhizosphere soil samples in the flowering period of peanuts in provinces such as Jilin, Liaoning, Jiangxi and Fujian were selected, and were named soil sample-1, soil sample-2, soil sample-3 and soil sample-4 in sequence, farmland soil samples to be tested were

sequentially weighed, after being uniformly crushed, the sample was transferred into the sample diluent in the kit at a concentration of 0.5g/mL, and the mixture was shaken to be uniform at room temperature to be prepared into a uniform dispersion of the sample to be tested, then 50μL of the uniform dispersion of the sample to be tested was taken and added into 30 mL of the Czapek-Dox medium in the kit for shaking culture at 28°C and at 200 rpm for 24 hours, and then sampling was carried out to form the solution to be tested of the sample to be identified.

Step 2, measurement of the solution to be tested of the sample to be identified: the solution to be tested was added into the wells of the ELISA plate in the kit in an amount of 100-200 μL per well, or the solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi of a series of concentrations in the kit was added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the solution was left at room temperature or at 37°C for reaction for no less than 1 hour, the post-reaction liquid was discarded, the ELISA plate was washed, and then the rabbit-derived polyclonal antibodies against AFT-YJFZP008 in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the rabbit-derived polyclonal antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the horseradish peroxidase-labeled goat anti-rabbit antibodies in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the horseradish peroxidase-labeled goat anti-rabbit antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the chromogenic solution for ELISA and the stop solution for ELISA in the kit were sequentially added, and finally the concentration of AFT-YJFZP008 in the sample to be tested was read and calculated by a microplate reader.

Step 3, evaluation on the identification results: if the concentration of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the sample to be identified is high, it is indicated that the sample to be identified contains an aflatoxin-producing strain with strong toxigenicity. According to the kit and the application technical scheme thereof, the measurement results of the concentrations of AFT-YJFZP008 in soil sample-1, soil sample-2, soil sample-3 and soil sample-4 were

as follows: 0.2 ng, 0.1 ng, 10.6 ng and 19.2 ng in sequence. The results showed that the amounts of AFT-YJFZP008 contained in each milliliter of culture solution in the soil sample-1 and the soil sample-2 were both less than 1.0 ng, indicating that the soil sample-1 and the soil sample-2 do not contain aflatoxin-producing strains with strong toxigenicity, so the risk of peanut post-harvest contamination in the farmland corresponding thereto was very low; and the amounts of AFT-YJFZP008 contained in each milliliter of culture solution in the soil sample-3 and the soil sample-4 were both more than 10 ng, indicating that the soil sample-3 and the soil sample-4 contain aflatoxin-producing strains with strong toxigenicity, so the risk of peanut post-harvest contamination in the farmland corresponding thereto was relatively high.

Example 9 Identification of whether there are aflatoxin-producing strains with strong toxigenicity in agricultural products using the rapid immunodetection kit for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

Step 1, preparation of solutions to be tested of samples to be identified: a total of four samples of agricultural products such as peanuts, corn, rice and wheat were selected, the amples to be tested were sequentially weighed, after being crushed, the sample was transferred into the sample diluent in the kit at a concentration of 0.5g/mL, and the mixture was shaken to be uniform at room temperature to be prepared into a uniform dispersion of the sample to be tested, 100μL of the uniform dispersion of the sample to be tested was taken and added into 50 mL of the Czapek-Dox medium in the kit for shaking culture at 28°C and at 200 rpm for 6 hours, and then sampling was carried out to form the solution to be tested of the sample to be identified.

Step 2, measurement of the solution to be tested of the sample to be identified: the solution to be tested was added into the wells of the ELISA plate in the kit in an amount of 100-200 μL per well, or the solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi of a series of concentrations in the kit was added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the solution was left at room temperature or at 37°C for reaction for no less than 1 hour, the post-reaction liquid was discarded, the ELISA plate was washed, and then the rabbit-derived polyclonal antibodies against AFT-YJFZP008 in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the rabbit-

derived polyclonal antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the horseradish peroxidase-labeled goat anti-rabbit antibodies in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the horseradish peroxidase-labeled goat anti-rabbit antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the chromogenic solution for ELISA and the stop solution for ELISA in the kit were sequentially added, and finally the concentration of AFT-YJFZP008 in a sample to be tested was read and calculated by a microplate reader.

Step 3, evaluation on the identification results: if the concentration of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the sample to be identified is high, it is indicated that the sample to be identified contains an aflatoxin-producing strain with strong toxigenicity. According to the kit and the application technical scheme thereof, the measurement results of the concentrations of AFT-YJFZP008 in peanut, corn, rice and wheat samples were 10.2 ng, 12.6 ng, 0.4 ng and 0 ng in sequence. The results showed that the amount of AFT-YJFZP008 contained in each milliliter of culture solution in the peanut sample and the corn sample was more than 10 ng, indicating that the peanut sample and the corn sample contain aflatoxin-producing strains with strong toxigenicity, so the risk of contamination was relatively high; the amount of AFT-YJFZP008 contained in each milliliter of culture solution in the rice sample was less than 1.0 ng, indicating that the rice sample does not contain an aflatoxin-producing strain with strong toxigenicity, so the risk of contamination was very low, and the amount of AFT-YJFZP008 contained in each milliliter of culture solution in the wheat sample was 0, indicating that in the wheat sample does not contain an aflatoxin-producing strain, so there was basically no risk of aflatoxin contamination.

Example 10 Identification of whether there are aflatoxin-producing strains with strong toxigenicity in traditional Chinese medicinal materials using the rapid immunodetection kit for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

Step 1, preparation of solutions to be tested of samples to be identified: a total of three samples of traditional Chinese medicinal materials such as ginseng, Chinese angelica

and wolfberry were selected, the traditional Chinese medicinal material samples to be tested were sequentially weighed, after being crushed, the sample was transferred into the sample diluent in the kit at a concentration of 0.5g/mL, and the mixture was shaken to be uniform at room temperature to be prepared into a uniform dispersion of the sample to be tested, then 50μL of the uniform dispersion of the sample to be tested was taken and added into 30 mL of the Czapek-Dox medium in the kit for shaking culture at 28°C and at 200 rpm for 6 hours, and then sampling was carried out to form the solution to be tested of the sample to be identified.

Step 2, measurement of the solution to be tested of the sample to be identified: the solution to be tested was added into the wells of the ELISA plate in the kit in an amount of 100-200 μL per well, or the solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi of a series of concentrations in the kit was added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the solution was left at room temperature or at 37°C for reaction for no less than 1 hour, the post-reaction liquid was discarded, the ELISA plate was washed, and then the rabbit-derived polyclonal antibodies against AFT-YJFZP008 in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the rabbit-derived polyclonal antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the horseradish peroxidase-labeled goat anti-rabbit antibodies in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the horseradish peroxidase-labeled goat anti-rabbit antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the chromogenic solution for ELISA and the stop solution for ELISA in the kit were sequentially added, and finally the concentration of AFT-YJFZP008 in a sample to be tested was read and calculated by a microplate reader.

Step 3, evaluation on the identification results: if the concentration of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the sample to be identified is high, it is indicated that the sample to be identified contains an aflatoxin-producing strain with strong toxigenicity. According to the kit and the application technical scheme thereof, the measurement results of the concentrations of

AFT-YJFZP008 in the samples of traditional Chinese medicinal materials such as ginseng, Chinese angelica and wolfberry were 0.2 ng/mL, 0 ng/mL and 0 ng/mL in sequence. The results showed that the measured concentration of AFT-YJFZP008 in the ginseng sample was less than 1.0 ng/mL, indicating that the ginseng sample does not contain an aflatoxin-producing strain with strong toxigenicity, so the contamination risk was very low; the measured concentrations of AFT-YJFZP008 in the Chinese angelica sample and the wolfberry sample were 0, indicating that the Chinese angelica sample and the wolfberry sample do not contain aflatoxin-producing strains, so there was basically no risk of aflatoxin contamination.

Example 11 Identification of whether there are aflatoxin-producing strains with strong toxigenicity in feed using the rapid immunodetection kit for the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi

Step 1, preparation of solutions to be tested of samples to be identified: a total of four feed samples to be tested were selected from the market and named feed-1, feed-2, feed-3 and feed-4 in sequence, the samples to be tested were sequentially weighed, after being crushed, the sample was transferred into the sample diluent in the kit at a concentration of 0.5g/mL, and the mixture was shaken to be uniform at room temperature to be prepared into a uniform dispersion of the sample to be tested, then 100μL of the uniform dispersion of the sample to be tested was taken and added into 200 mL of the Czapek-Dox medium in the kit for shaking culture at 28°C and at 200 rpm for 24 hours, and then sampling was carried out to form the solution to be tested of the sample to be identified.

Step 2, measurement of the solution to be tested of the sample to be identified: the solution to be tested was added into the wells of the ELISA plate in the kit in an amount of 100-200 μL per well, or the solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi of a series of concentrations in the kit was added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the solution was left at room temperature or at 37°C for reaction for no less than 1 hour, the post-reaction liquid was discarded, the ELISA plate was washed, and then the rabbit-derived polyclonal antibodies against AFT-YJFZP008 in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the rabbit-derived polyclonal antibodies were left at room temperature or at 37°C for reaction for

no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the horseradish peroxidase-labeled goat anti-rabbit antibodies in the kit were added into the wells of the ELISA plate in an amount of 100-200 μL per well, after the horseradish peroxidase-labeled goat anti-rabbit antibodies were left at room temperature or at 37°C for reaction for no less than 1 hour, the liquid was discarded, and then the ELISA plate was washed again, then the chromogenic solution for ELISA and the stop solution for ELISA in the kit were sequentially added, and finally the concentration of AFT-YJFZP008 in a sample to be tested was read and calculated by a microplate reader.

Step 3, evaluation on the identification results: if the concentration of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the sample to be identified is high, it is indicated that the sample to be identified contains an aflatoxin-producing strain with strong toxigenicity. According to the kit and the application technical scheme thereof, the measurement results of the concentrations of AFT-YJFZP008 in feed-1, feed-2, feed-3 and feed-4 were: 0.5 ng, 20.3 ng, 14.2 ng and 72.9 ng in sequence. The results showed that the amount of AFT-YJFZP008 contained in each milliliter of culture solution in the sample of feed-1 was less than 1.0 ng/mL, indicating that the sample of feed-1 does not contain an aflatoxin-producing strain with strong toxigenicity, so the contamination risk was very low; taking 50 μL of sample uniform dispersion added to a 30 mL of medium as an example, the amounts of AFT-YJFZP008 contained in each milliliter of culture solution in the samples feed-2, feed-3 and feed-4 were all more than 10 ng/mL, indicating that the samples feed-2, feed-3 and feed-4 contain aflatoxin-producing strains with strong toxigenicity.

CLAIMS

1. A kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi, characterized by comprising: an ELISA plate coated with nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi at the bottoms of wells, polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, a pure solution of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi functioning as a reference material, a Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus*, horseradish peroxidase-labeled antibodies that bind to the polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, a chromogenic solution for ELISA, a stop solution for ELISA, and a sample diluent;

the molecule indicating the toxigenicity of the aflatoxin-producing fungi refers to an AFT-YJFZP008 peptide, of which the amino acid sequence is shown in SEQ ID NO. 1.

2. The kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi according to claim 1, characterized in that the ELISA plate coated with nanobodies or monoclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi at the bottoms of wells is prepared by the following method: dissolving the nanobodies or monoclonal antibodies against AFT-YJFZP008 in ELISA coating buffer to form a coating solution of 0.2-8.0 μg/mL, then adding the coating solution into the ELISA plate, after leaving the coating solution at 4°C overnight or at 37°C for no less than 2 hours, removing the coating solution in the ELISA plate, then washing the ELISA plate with a conventional washing solution for ELISA; then adding a conventional blocking solution for ELISA into the ELISA plate, after leaving the blocking solution at room temperature or at 37°C for blocking for no less than 1 hour, discarding the blocking solution, and then washing the ELISA plate with the conventional washing solution for ELISA.

3. The kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi according to claim 1, characterized in that the polyclonal

antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi are rabbit-derived polyclonal antibodies against the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi, and the horseradish peroxidase-labeled antibodies are horseradish peroxidase-labeled goat anti-rabbit antibodies.

4. The kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi according to claim 1, characterized in that the chromogenic solution for ELISA refers to a conventional hydrogen peroxide and TMB chromogenic solution for ELISA;

the stop solution refers to a conventional color development stop solution for ELISA: 2 mol/L sulfuric acid aqueous solution.

5. The kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi according to claim 1, characterized in that the sample diluent refers to 0.01 mol/L phosphate buffer containing 0.1% sorbitol and 0.1% sugar.

6. A method of using the kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi according to claim 1, comprising the following steps:

culturing a strain to be identified or a sample to be identified with the Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus* in the kit, and preparing a solution to be tested of the strain to be identified or a sample to be identified;

diluting the solution to be tested of the strain to be identified or the sample to be identified with the sample diluent, adding the diluted solution to be tested of the strain to be identified or the sample to be identified into the wells of the ELISA plate in the kit in an amount of 100-200 μL per well, after leaving the diluted solution at room temperature or at 37°C for reacting for no less than 1 hour, discarding the post-reaction liquid, and washing the ELISA plate;

then adding the polyclonal antibodies against AFT-YJFZP008 in the kit into the wells of the ELISA plate in an amount of 100-200 μL per well, after leaving the

polyclonal antibodies at room temperature or at 37°C for reacting for no less than 1 hour, discarding the liquid, and then washing the ELISA plate;

then adding the horseradish peroxidase-labeled antibodies in the kit into the wells of the ELISA plate in an amount of 100-200 μL per well, after leaving the horseradish peroxidase-labeled antibodies at room temperature or at 37°C for reacting for no less than 1 hour, discarding the liquid, and then washing the ELISA plate; and

then adding the chromogenic solution for ELISA and the stop solution for ELISA in the kit successively, and finally reading and calculating the concentration of AFT-YJFZP008 in the sample to be tested by a microplate reader.

7. The method according to claim 6, characterized in that the solution to be tested is replaced with the pure solution, of a series of concentrations, of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi functioning as a reference material for making a standard curve for calculating the concentration of AFT-YJFZP008 in the sample to be tested.

8. The method according to claim 6, characterized by further comprising: evaluating the detection results: obtaining the content of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the solution to be tested of the strain to be identified in unit volume according to results displayed by the microplate reader, and analyzing the aflatoxin-producing ability of the strain to be identified, wherein the higher the concentration of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the strain to be identified, the stronger the aflatoxin-producing ability, that is, the toxigenicity of the strain to be identified; and

obtaining the content of the molecule AFT-YJFZP008 indicating the toxigenicity of the aflatoxin-producing fungi in the solution to be tested of the sample in unit volume according to the results displayed by the microplate reader, and judging whether the sample to be identified contains an aflatoxin-producing strain with strong toxigenicity.

9. The method according to claim 6, characterized in that the preparation of the solution to be tested of the strain to be identified comprises: culturing the strain to be identified, carrying out dilution to obtain the solution to be tested of the strain to be

identified, specifically, culturing the strain to be identified in a conventional Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus* at an ambient temperature of 15-35°C for no less than 12 hours, then fully homogenizing a mixture of the culture medium and the culture, and then diluting the mixture 1-10 times with sterile water to obtain the solution to be tested of the strain to be identified;

the preparation of the sample solution to be identified comprises: culturing the sample to be identified to obtain the solution to be tested of the sample to be identified, specifically, weighing the sample to be identified, transferring the sample to be identified into the sample diluent, shaking the obtained mixture at room temperature until the mixture is uniform to prepare a uniform dispersion of the sample to be tested, adding 10-1,000 μL of the uniform dispersion of the sample into a 6-600 mL conventional Czapek-Dox medium or other culture mediums suitable for the growth of toxigenic *Aspergillus flavus* for shaking culture at 15-35°C and at 200±50 rpm for 6-24 hours, and then carrying out sampling to obtain the solution to be tested of the sample to be identified.

10. Application of the kit for rapid detection of a molecule indicating the toxigenicity of the aflatoxin-producing fungi according to claim 1 to the identification of the aflatoxin-producing ability of the strains of genus *Aspergillus* or the identification of whether there are aflatoxin-producing strains with strong toxigenicity in farmland, agricultural products and feed samples, wherein the samples are farmland, agricultural products, traditional Chinese medicinal materials and feed.