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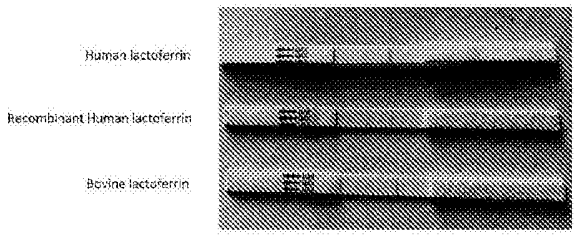
(54) **TEST STRIP FOR DOUBLE-ANTIBODY SANDWICH COLLOIDAL GOLDDETECTION AGAINST HUMAN LACTOFERRIN**

(57) The invention provides a test strip for double-antibody sandwich colloidal gold detection against human lactoferrin, the test strip comprises a water absorption pad, a base film, a gold-labeled pad and a sample pad which are connected in sequence; a line C and a line T are arranged on the base film, the line C is coated with an antibody of goat anti-mouse IgG, and the gold-labeled pad contains a first monoclonal antibody labeled with colloidal gold; the line T is coated with a second monoclonal antibody; the first monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10595, and the second monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10594. The detection method with immune test strip of double-antibody sandwich can be used for detecting human lactoferrin, with the sensitivity of 10ng/mL, wherein no cross reaction for bovine lactoferrin and goat lactoferrin is presented, and no cross reaction for lysozyme transgenic cattle and human alpha-serum albumin transgenic cattle is presented.



TEST STRIP FOR DOUBLE-ANTIBODY SANDWICH COLLOIDAL GOLD DETECTION AGAINST HUMAN LACTOFERRIN

Field of the Invention

The present invention relates to the field of biotechnology, and particularly, relates to a test strip for double-antibody sandwich colloidal gold detection against human lactoferrin.

Background of the Invention

"Big rat" came out (Palmiter et al., 1982), and accelerated the research of transgenic organisms. The birth of clo

ned sheep "Dolly" (Wilmut et al., 1997) promotes wide application of transgenic technology in mammals. Various transgenic pigs, cattle and sheep (Schnieke et al., 1997; Toledo et al., 2006) have been successfully researched at present for producing medicinal or edible protein, improving the lean meat rate, improving the nutrition and strengthening the disease resistance, which have huge potential economic benefits and social benefits and good commercial prospect. However, since the transgenic organisms and products thereof have risk, their management gains high attention from national governments all the time.

In order to protect the health and the living environment of human beings, many countries including China have successively released the transgenic organism safety laws and regulations, adopted the labeling systems for transgenic organisms and products, and strictly managed the import and export of transgenic food. It needs valid detection technologies to support successful implementation of the laws, regulations and systems. The present detection methods for transgenic organisms and processed food thereof mainly include gene-level detection and protein-level detection, the detection method for human lactoferrin is mainly based on ELISA in protein detection at present, but the ELIS method having long operation time and needing professional equipment such as an ELISA instrument is not suitable for on-site quick detection. Hence, it is very necessary to establish a quick and convenient detection method.

The present method most suitable for on-site detection is mainly immune colloidal gold technology, which is a novel immune labeling technology applying colloidal gold to antigens and antibodies as a tracing marker. The colloidal gold is formed by aggregating chloroauric acid ($HAuCl_4$) into gold particles with specific size in the presence of a

reducer such as ascorbic acid, sodium citrate, tannic acid and the like, and processing the gold particles into a stable colloidal state due to electrostatic interaction. The colloidal gold carries negative charges in a weak alkaline environment and can be firmly bonded with positive charge groups of protein molecules, and such bonding is electrostatic bonding and thus does not influence the biological characteristics of protein. The colloidal gold is widely applied in the fields of immunology, histology, pathology, cytobiology and the like according to some physical properties of the colloidal gold, such as high electron density, particle size, shape and color reaction, as well as immune and biological characteristics of conjugates.

A double-antibody sandwich test strip for detecting human lactoferrin can realize quick and convenient detection of the human lactoferrin. However, existing antibodies against human lactoferrin cannot meet the requirements of the double-antibody sandwich immune test strip for detecting human lactoferrin due to low sensitivity and specificity and poor pairing property.

Summary of the Invention

An object of the present invention is to provide a high-sensitive and high-specific test strip for double-antibody sandwich colloidal gold detection against human lactoferrin, which can meet the requirements of double-antibody sandwich immune test strip detection for human lactoferrin and realize quick and convenient detection of the human lactoferrin.

To fulfill the above object, the present invention provides a test strip for double-antibody sandwich colloidal gold detection against human lactoferrin, the said test strip comprises a water absorption pad, a base film, a gold-labeled pad and a sample pad which are connected in sequence; a line C and a line T are arranged on the base film, the line C is coated with an antibody of goat anti-mouse IgG, and the gold-labeled pad contains a first monoclonal antibody labeled with colloidal gold; the line T is coated with a second monoclonal antibody; the first monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10595, and the second monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10594.

Through the above technical solution, the detection method with immune test strip of double-antibody sandwich can be used for detecting human lactoferrin, with the sensitivity of 10ng/mL, wherein no cross reaction for bovine lactoferrin and goat lactoferrin is presented, no cross reaction for human lysozyme transgenic cattle and

human α-serum albumin transgenic cattle is presented, and the test strip has high sensitivity and specificity.

Other features and advantages of the present invention will be described in detail in the following specific implementation part.

Deposition of biological materials

One hybridoma cell strain of the present invention is obtained by inventor's voluntary hybridization screening, whose deposit number is CGMCC NO.10595, whose deposit date is May 8, 2015, whose depositary institution is China General Microbiological Culture Collection Center, and its address is Institute of Microbiology of Chinese Academy of Science, No. 3, Yard 1, West Beichen Road, Chaoyang District, Beijing; whose scientific name is a hybridoma cell strain.

The other hybridoma cell strain of the present invention is obtained by inventor's voluntary hybridization screening, whose deposit number is CGMCC NO.10594, whose deposit date is May 8, 2015, whose depositary institution is China General Microbiological Culture Collection Center, and its address is Institute of Microbiology of Chinese Academy of Science, No. 3, Yard 1, West Beichen Road, Chaoyang District, Beijing; whose scientific name is a hybridoma cell strain.

Brief Description of the Drawings

The accompanying drawings constituting a part of the specification are used for providing further understanding of the present invention, and interpreting the present invention together with the following specific embodiment, rather than limiting the present invention. In which:

Fig. 1 is a structural schematic diagram of an immune colloidal gold test strip.

Fig. 2 is a schematic diagram of a theoretical detection result of the test strip.

Fig. 3 is a detection diagram of specificity test of a test strip.

Fig. 4 is a detection diagram of part of positive samples.

Fig. 5 is a detection diagram of part of negative samples

Reference signs:

1 sample pad 2 colloidal gold pad 3 base film

4 absorption pad 5 line T 6 line C

Detailed Description of the Embodiments

The specific embodiments of the present invention will be described in detail below in combination with the accompanying drawings. It should be understood that the specific embodiments described herein are merely used for illustrating and interpreting the present invention, rather than limiting the same.

The present invention provides test strip for double-antibody sandwich colloidal gold detection against human lactoferrin, the said test strip comprises a water absorption pad, a base film, a gold-labeled pad and a sample pad which are connected in sequence; a line C and a line T are arranged on the base film, the line C is coated with an antibody of goat anti-mouse IgG, and the gold-labeled pad contains a first monoclonal antibody labeled with colloidal gold; the line T is coated with a second monoclonal antibody; the first monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10595, and the second monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10594.

The detection principle of the immune colloidal gold test strip may include: a sample to be detected is dropped into the sample pad; if the sample contains human lactoferrin, the human lactoferrin in the sample to be detected is bonded with the colloidal gold labeled first monoclonal antibody (3B4) first; the complex formed by the human lactoferrin and the colloidal gold labeled first monoclonal antibody swims forward along the base film due to the capillary action, and encounters the second monoclonal antibody (5C5) coated on the cellulose nitrate base film when arriving at a detection line; the first monoclonal antibody and the second monoclonal antibody can be separately bonded with different epitopes of human lactoferrin to form a "colloidal gold labeled first monoclonal antibody (3B4)-human lactoferrin-second monoclonal antibody (5C5)" complex, so that colloidal gold is enriched on the detection line to form a specific red chromatographic line; the colloidal gold labeled first monoclonal antibody not bonded with the human lactoferrin directly passes through the detection line, arrives at a quality control line and then is captured by goat anti-mouse IgG on the quality control line, so that colloidal gold is enriched on the quality control line to form a red chromatographic line, which is judged as a positive result; if the sample does not contain human lactoferrin, the "colloidal gold labeled first monoclonal antibody (3B4)-human lactoferrin-second monoclonal antibody (5C5)" complex cannot be formed on the detection line, so that macroscopic color reaction is not produced on the detection line, wherein the colloidal gold labeled first monoclonal antibody not bonded with human lactoferrin directly passes through the

detection line, arrives at the quality control line and then is captured by goat anti-mouse IgG on the quality control line, so that colloidal gold is enriched on the quality control line to form a red chromatographic line, which is judged as a negative result.

In the present invention, the methods for separately preparing and purifying the first monoclonal antibody and the second monoclonal antibody by using the hybridoma cell strain with the collection number of CGMCC NO.10595 and the hybridoma cell strain with the collection number of CGMCC NO.10594 may be conventional methods in the art, such as a mouse ascites method and an affinity chromatography method.

In the present invention, the colloidal gold labeling method for the monoclonal antibody may be a conventional method in the art, for example, including the following steps: heating 0.01wt% of $HAuCl_4$ solution to boil, adding 1wt% of trisodium citrate solution till the solution is completely transparent red, continuously performing reflux for 10min and then stopping heating, cooling the solution to room temperature, thus obtaining colloidal gold; adjusting the pH value of 1ml of the prepared colloidal gold to 8.0 with 1wt% of K_2CO_3, adding 8μg of monoclonal antibody, mixing uniformly, and reacting for 40min at room temperature; adding 5wt% of BSA till the final concentration is 0.1wt%, and standing for 30min; performing low-speed (1500r/min) centrifuging for 15min to remove precipitate formed by condensed colloidal gold particles; then centrifuging for 30min at 10000r/min; carefully sucking the supernate off, re-dissolving the precipitate with 0.1ml of 0.1M PBS (pH7.4) containing 1% BSA, adding 5% of sodium azide till the final concentration is 0.05%, and preserving at 4□.

In the present invention, the method for preparing the immune colloidal gold test strip may be a conventional method in the art, for example, including the following steps: spraying the second monoclonal antibody and the goat anti-mouse IgG onto the cellulose nitrate film by using a gold-spraying film dispensing machine to form a detection line T and a quality control line C which are parallel to each other, and then drying the cellulose nitrate film; uniformly spraying the colloidal gold labeled first monoclonal antibody onto the gold-labeled pad by using the gold-spraying film dispensing machine; and then connecting and assembling the sample pad, the gold-labeled pad, the base film (cellulose nitrate film) sprayed with the line T and the line C and a piece of water absorption paper in sequence, cutting and packaging for standby use.

The first monoclonal antibody labeled with colloidal gold contained in the gold-labeled pad may be obtained by spraying a solution containing 5-100μg/mL of the first monoclonal antibody labeled with colloidal gold at the spraying rate of 0.5-5μL/cm on

the gold-labeled pad via a gold-spraying film dispensing machine and then drying the gold-labeled pad.

The second monoclonal antibody coated onto the line T may be obtained by spraying a solution containing 0.2-5mg/mL of second monoclonal antibody at the spraying rate of 0.2-4μL/cm on the line T via a gold-spraying film dispensing machine and then drying the line T.

The the goat anti-mouse IgG coated onto the line C may be obtained by spraying a solution containing 0.2-5mg/mL of the goat anti-mouse IgG antibody at the spraying rate of 0.2-4μL/cm on the line C via the gold-spraying film dispensing machine and then drying the line C.

The present invention will be further described in detail below via embodiments:

Embodiment 1

This embodiment is used for describing screening of an antibody pair for the double-antibody sandwich colloidal gold detection test strip for human lactoferrin.

The protein sequence of the human lactoferrin (hLF) is shown as the NCBI Genbank sequence number: U95626. Transgenic dairy cattle of human lactoferrin is obtained according to the method in the document (Penghua Yang, et al., Cattle Mammary Bioreactor Generated by a Novel Procedure of Transgenic Cloning for Large-Scale Production of Functional Human Lactoferrin., PLoS ONE, 2008, 3(10), e3453).

The muscle of the transgenic dairy cattle is injected with lactogenic injection (available from infertile cattle lactogenic injection produced by Xi'an Caotan Pharmaceutical Factory, the product is boxed, 10 pieces of No. I injection and 3 pieces of No. II injection are needed) to promote lactation, wherein the muscle is continuously injected with the No. I injection for 10 days once every day. The muscle is injected with one piece of No. II injection respectively at the 13^{th}, 15^{th} and 17^{th} days. The breasts of the transgenic dairy cattle are massaged with warm water every day from 7 days of injection, and hand milking is performed for two weeks twice every day after the No. II injection is injected to obtain a milk sample.

The milk sample is centrifuged for 15min at 4□ and 4500rpm and then taken out, solidified milk fat is on the upper layer, and the lower layer of liquid is sucked off and transferred to another centrifugal tube. The pH value of the skimmed milk is adjusted to 3.8-4.6 with 1M of hydrochloric acid, so that casein is precipitated, wherein the volume ratio of the skimmed milk to the 1M of HCl is 25: 1; and then the milk sample with the

adjusted pH value is centrifuged for 12min at 4□ and 5000rpm. The milk sample is taken out, wherein the precipitate is casein, and the supernate is whey protein; and the supernate is transferred to another centrifugal tube. The pH value of the supernate is adjusted back to 7.5 with 1M of NaOH aqueous solution, wherein the volume ratio of the supernate to the 1M of NaOH aqueous solution is 25: 1. The milk sample with the adjusted pH value is centrifuged for 15min at 4□ and 5000rpm, and the centrifuged supernate is collected to obtain purified whey.

A HiLoadTM 16/10SP Sepharose High performance chromatographic column are balanced by previously using 5 column volumes of solution containing 0.4M of NaCl and 20mM of Na_3PO_4(pH7.5). 15ml of skimmed milk is injected to the sample by an injector, and enters a sample loading ring. Column is balanced by 5 column volumes of solution containing 0.4M of NaCl and 20mM of Na_3PO_4(pH7.5), the unbounded protein is collected into a centrifugal tube, column is eluted by 15 column volumes of solution containing 0.4M-1M of NaCl and 20mM of Na_3PO_4(pH7.5), and the separated protein of each peak is collected and the elutes of the same peak are combined. Column is continuously eluted by 2 column volumes of solution containing 1M of NaCl and 20mM of Na_3PO_4(pH7.5), and column is finally eluted by 5 column volumes of solution containing 1M of NaCl and 20mM of Na_3PO_4(pH7.5). The above operations are all performed on an AKTA purifer10 liquid chromatograph. An elution peak at 0.6M of NaCl is finally obtained. The elute at the elution peak is collected and subjected to ultra-filtration concentration and freeze drying, and then the elution peak is proved as recombinant purified human lactoferrin (hLF) by N-end sequencing and protein electrophoresis.

A female BALB/c mouse is immunized by using the purified human lactoferrin (hLF), spleen cells and myeloma cells are fused, and 10 cloned cell strains for stably secreting hLF monoclonal antibodies are obtained by positive clone screening via an ELISA method and sub-cloning via a limited dilution method. The hybridoma cell strains are respectively injected to the abdominal cavity of the mouse, ascites is collected, and 10 kinds of monoclonal antibodies are obtained by affinity column purification.

Every two of the 10 kinds of monoclonal antibodies are combined to obtain 45 combinations, each combination is prepared into a colloidal gold immune test strip, specifically, one antibody in each combination is labeled with colloidal gold and prepared into a gold-labeled pad, while the other antibody is used for preparing a line T.

A preparation method of the colloidal gold includes the following steps: heating 0.01wt%

of $HAuCl_4$ solution to boil, adding 1wt% of trisodium citrate solution till the solution is completely transparent red, continuously performing reflux for 10min and then stopping heating, cooling the solution to room temperature, thus obtaining colloidal gold. A colloidal gold labeling method for the monoclonal antibody comprises the following steps: adjusting the pH value of 1ml of the prepared colloidal gold to 8.0 with 1wt% of K_2CO_3, adding 8μg of monoclonal antibody, mixing uniformly, and reacting for 40min at room temperature; adding 5wt% of BSA till the final concentration is 0.1wt%, and standing for 30min; performing low-speed (1500r/min) centrifuging for 15min to remove precipitate formed by condensed colloidal gold particles; then centrifuging for 30min at 10000r/min; carefully sucking the supernate off, re-dissolving the precipitate with 0.1ml of 0.1M PBS (pH7.4) containing 1% of BSA, adding 5% of sodium azide till the final concentration is 0.05%, and preserving at 4□.

The second monoclonal antibody and the goat anti-mouse IgG are sprayed onto the cellulose nitrate film by using a gold-spraying film dispensing machine to form a detection line T and a quality control line C which are parallel to each other, and then the cellulose nitrate film is dried; the colloidal gold labeled first monoclonal antibody is uniformly sprayed onto the gold-labeled pad by using the gold-spraying film dispensing machine; and then as shown in Fig. 1, the sample pad, the gold-labeled pad, the base film (cellulose nitrate film) sprayed with the line T and the line C and a piece of water absorption paper are connected and assembled in sequence, and the assembly is cut and packaged for later use. Totally 45 kinds of immune colloidal gold test strips are obtained. For the gold-labeled pad, the spraying speed of the gold-spraying film dispensing machine is 2.0μL/cm. For the line T and the line C, the spraying speed of the gold-spraying film dispensing machine is 1μL/cm, the line T is 1mg/mL of second monoclonal antibody, and the line C is 1mg/mL of goat anti-mouse IgG antibody.

Said 45 kinds of immune colloidal gold test strips are screened, using the PBS solution of human lactoferrin as a positive sample and using the PBS solution of bovine lactoferrin as a negative sample. 44 kinds of immune colloidal gold test strips having negative reaction to the human lactoferrin or having positive reaction to the bovine lactoferrin are removed, thus one immune colloidal gold test strip is finally obtained, which has positive reaction to 10ng/mL of human lactoferrin PBS solution but does not have the positive reaction to 100μg/mL of bovine lactoferrin PBS solution. This immune colloidal gold test strip is the one screened in this embodiment. The first monoclonal antibody for the immune colloidal gold test strip is secreted by a hybridoma cell strain 3B4 with the

collection number of CGMCC NO.10595; and the second monoclonal antibody for the immune colloidal gold test strip is secreted by a hybridoma cell strain 5C5 with the collection number of CGMCC NO.10594. The subtypes of the two monoclonal antibodies are IgG1+kappa upon identification.

Embodiment 2

This embodiment is used for describing the sensitivity and the specificity of the double-antibody sandwich colloidal gold detection test strip for human lactoferrin (i.e., the immune colloidal gold test strip screened in embodiment 1) when an actual sample is tested.

(1) Sample preparation: collected milk, dissolved milk powder and a liquid dairy product. About 200μl of milk sample is added into a sample tube.

(2) Detection: the immune colloidal gold test strip screened in embodiment 1 is balanced for 20min at room temperature, and the test strip is unpackaged, inserted into the sample tube in the arrow direction and stood for 10-15min to obtain a judgment result.

(3) Result judgment: when a macroscopic purple red quality control line appears in the test strip but a macroscopic purple red detection line does not appear, it is judged as negative and marked as "-"; when both the macroscopic purple red quality control line and the macroscopic purple red detection line appear in the test strip, it is judged as positive and marked as "+"; the color depth of the detection line is directly proportional to the quantity of an antigen to be detected; the deeper the color is, the higher the quantity of the antigen to be detected is; and if the quality control line is not shown, the test strip is judged as invalid. The schematic diagram of the judgment results is shown in Fig. 2.

Table 1 shows the detection results of specificities of PBS solution of a purchased Sigma human lactoferrin standard (purified), PBS solution of a human lactoferrin standard (recombinant expression), PBS solution of a purchased Sigma bovine lactoferrin standard, PBS solution of a purchased Sigma goat lactoferrin standard, PBS solution of a purchased Sigma human lysozyme standard and PBS solution of a purchased Sigma human α-serum albumin standard. The results show that the test strip can specifically identify human lactoferrin, but does not have positive reaction to bovine lactoferrin, goat lactoferrin, human lysozyme and human α-serum albumin. Part of the detection results are shown in Fig. 3.

Table 1 Specificity test results of the test strip

Sample	Human lactoferrin standard (purified)	Human lactoferrin standard (recombinant expression)	Bovine lactoferrin standard	Goat lactoferrin standard	Human lysozyme standard	Human α-serum albumin standard
Detection Result	+	+	-	-	-	-

Gradient dilution is performed on the human lactoferrin standard (purified) and the human lactoferrin standard (recombinant expression), the sensitivities are detected with the colloidal gold test strip, the detection results are shown in table 2, and the results of table 2 show that the sensitivities of the test strip can reach 10ng/mL.

Table 2 Sensitivity test results of the test strip

Dilution / Standard	1 mg/mL	100 μg/mL	10 μg/mL	1 μg/mL	100 ng/mL	10 ng/mL	1 ng/mL
Human lactoferrin standard (purified)	+	+	+	+	+	+	-
Human lactoferrin standard (recombinant expression)	+	+	+	+	+	+	-

Embodiment 3

140 samples of milk containing known negative human lactoferrin (including 100 samples of milk collected from non-transgenic dairy cattle in the Dairy Farm of Hebei Big Plant, 20 samples of milk from human lysozyme transgenic dairy cattle in the Dairy Farm of China Agricultural University and 20 samples of milk from human α-serum albumin transgenic dairy cattle in the Dairy Farm of China Agricultural University) and

22 samples of milk containing known positive human lactoferrin (collected from human lactoferrin transgenic dairy cattle in the Dairy Farm of China Agricultural University), namely 162 samples in total, are collected, and are blindly detected with the immune colloidal gold test strip screened in embodiment 1. The detection results show that the 140 samples of milk containing known negative human lactoferrin are negative, the 22 samples of milk containing known positive human lactoferrin are positive, and the diagnostic specificity of the test strip is 100%. Part of the detection results are shown in Fig. 4 and Fig. 5.

It can be seen from the data of embodiments 1-3 that the double-antibody sandwich colloidal gold detection test strip can be used for detecting human lactoferrin by a double-antibody sandwich immune test strip detection method, so that the sensitivity of 10ng/mL can be achieved, no cross reaction for bovine lactoferrin and goat lactoferrin is caused, no cross reaction for lysozyme transgenic cattle and human α-serum albumin transgenic cattle is caused, and the test strip has high sensitivity and specificity. This may be because the monoclonal antibodies used by the double-antibody sandwich colloidal gold detection test strip for human lactoferrin in the present invention can identify the human lactoferrin with high sensitivity and high specificity, and the two monoclonal antibodies of the present invention can be separately directed against different antigenic determinants and thus are particularly suitable for detection of the double-antibody sandwich immune test strip.

The preferred embodiments of the present invention are described in detail above in combination with the accompanying drawings, but the present invention is not limited to the specific details in the aforementioned embodiments, many simple modifications may be made to the technical solutions of the present invention within the technical conception scope of the present invention, and these simple modifications fall into the protection scope of the present invention.

In addition, it should be noted that the specific technical features described in the above specific embodiments can be combined in any proper mode without conflicts, and in order to avoid unnecessary repetition, various possible combination modes are not additionally described in the present invention.

Moreover, various different embodiments of the present invention can also be randomly combined without departing from the thought of the present invention, and these combinations should be regarded as the contents disclosed by the present invention likewise.

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CONCLUSIES

1. Teststrip voor dubbel-antilichaam sandwich colloïdale gouddetectie tegen humaan lactoferrine, de betreffende teststrip omvattende een waterabsorptiepad, een basisfilm, een goud-gelabelde pad en een monsterpad die sequentieel verbonden zijn, waarbij een lijn C en een lijn T gepositioneerd zijn op de basisfilm, de lijn C gecoat is met een antilichaam geit anti-muis IgG, en de goud-gelabelde pad een eerste monoklonaal antilichaam bevat gelabeld met colloïdaal goud; de lijn T gecoat is met een tweede monoklonaal antilichaam; het eerste monoklonaal antilichaam uitgescheiden is door een hybridomacellijn met het depotnummer CGMCC NO.10595, en het tweede monoklonaal antilichaam uitgescheiden is door een hybridomacellijn met het depotnummer CGMCC NO.10594.

2. Teststrip voor dubbel-antilichaam sandwich colloïdale gouddetectie tegen humaan lactoferrine volgens conclusie 1, waarbij het eerste monoklonaal antilichaam dat gelabeld is met colloïdaal goud, dat zit in de goud-gelabelde pad, verkregen is door het sproeien van een oplossing bevattend 5-100 μg/mL van het eerste monoklonaal antilichaam dat gelabeld is met colloïdaal goud, bij de sproeisnelheid van 0.5-5 μL/cm op de goud-gelabelde pad door middel van een goud-sproeiende filmdoseermachine en vervolgens het drogen van de goud-gelabelde pad.

3. Teststrip voor dubbel-antilichaam sandwich colloïdale gouddetectie tegen humaan lactoferrine volgens conclusie 1, waarbij het tweede monoklonaal antilichaam gecoat op de lijn T verkregen is door een oplossing te sproeien die 0.2-5 mg/mL van het tweede monoklonaal antilichaam bevat, bij de sproeisnelheid van 0.2-4 μL/cm op de lijn T door middel van een goud-sproeiende filmdoseermachine en vervolgens het drogen van de lijn T.

4. Teststrip voor dubbel-antilichaam sandwich colloïdale gouddetectie tegen humaan lactoferrine volgens conclusie 1, waarbij het geit anti-muis IgG gecoat op de lijn C verkregen is door een oplossing te sproeien die 0.2-5 mg/mL van het geit anti-muis IgG antilichaam bevat, bij de sproeisnelheid van 0.2-4 μL/cm op de lijn C door middel van een goud-sproeiende filmdoseermachine en vervolgens het drogen van de lijn C.

Drawings

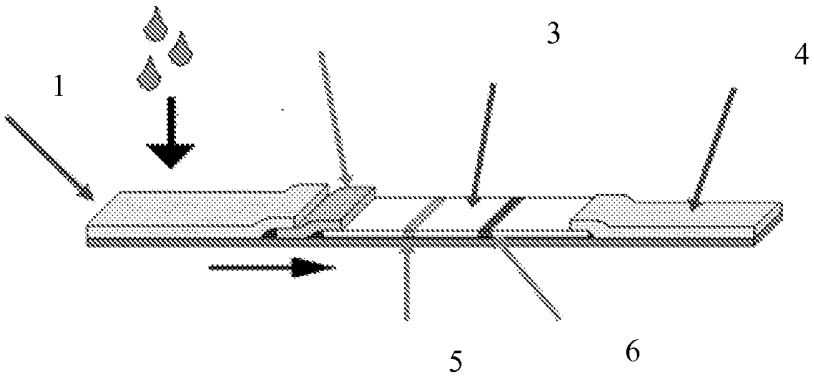

Fig. 1

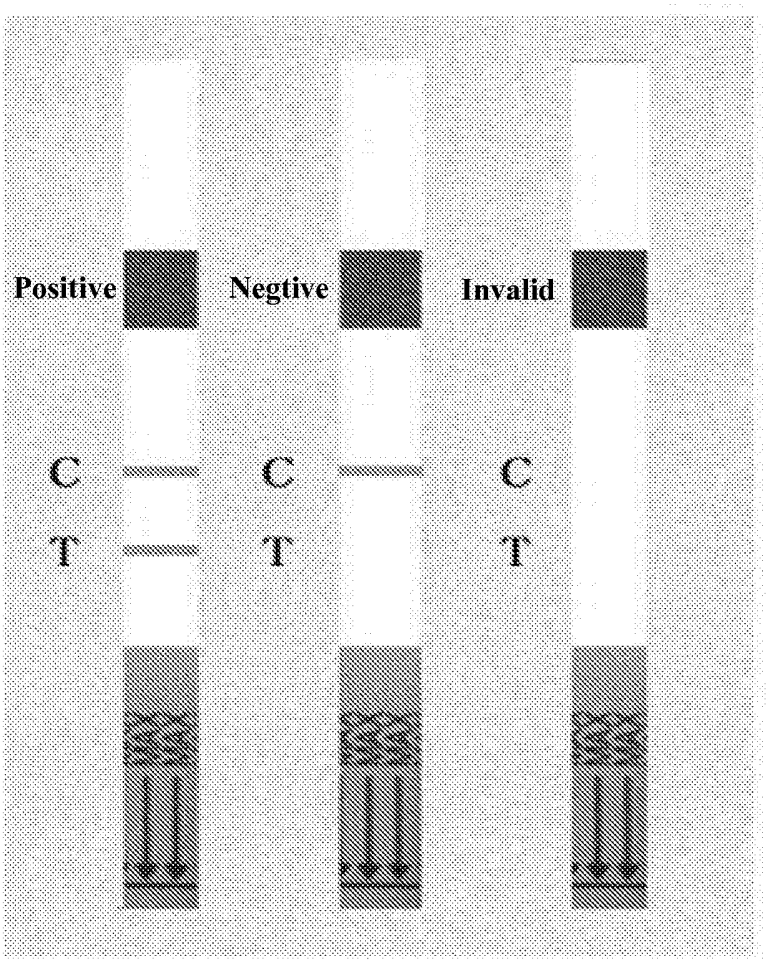

Fig. 2

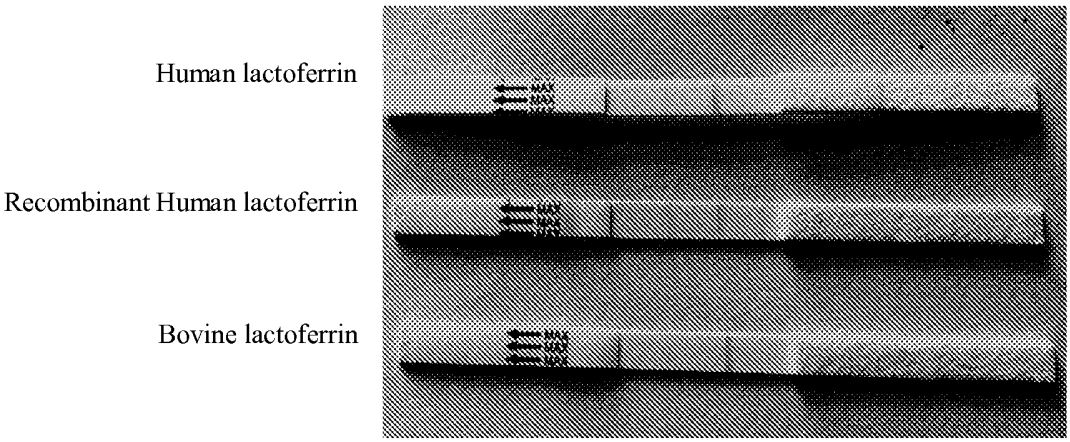
Human lactoferrin
Recombinant Human lactoferrin
Bovine lactoferrin

Fig. 3

Fig. 4

Fig. 5

Abstract

The invention provides a test strip for double-antibody sandwich colloidal gold detection against human lactoferrin, the test strip comprises a water absorption pad, a base film, a gold-labeled pad and a sample pad which are connected in sequence; a line C and a line T are arranged on the base film, the line C is coated with an antibody of goat anti-mouse IgG, and the gold-labeled pad contains a first monoclonal antibody labeled with colloidal gold; the line T is coated with a second monoclonal antibody; the first monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10595, and the second monoclonal antibody is secreted by a hybridoma cell strain with the deposit number of CGMCC NO.10594. The detection method with immune test strip of double-antibody sandwich can be used for detecting human lactoferrin, with the sensitivity of 10ng/mL, wherein no cross reaction for bovine lactoferrin and goat lactoferrin is presented, and no cross reaction for lysozyme transgenic cattle and human alpha-serum albumin transgenic cattle is presented.

Figure 3.