

Development of an Indirect Competitive ELISA Kit for the Detection of Soybean Allergenic Protein Gly m Bd 28K

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Abstract The complementary DNA (cDNA) sequence of Gly m Bd 28K was expressed in *Escherichia coli* BL21 (DE3) as an inclusion body under the induction of 1.0 mmol/L of isopropyl β -D-thiogalactoside (IPTG). The inclusion bodies were solubilized in 6 M guanidine hydrochloride and refolded by rapid dilution. After renaturation, the purity of the recovered recombinant protein was up to 90 %, and its molecular weight was 29.1 kDa. A monoclonal antibody that was specific for recombinant Gly m Bd 28K protein was produced and characterized. Based on the mAb, an indirect competitive enzyme-linked immunosorbent kit (icELISA kit) for the specific detection of recombinant Gly m Bd 28K protein was developed, which showed that the IC₅₀ was 3.19 μ g/L and the sensitivity was 0.235 μ g/L. The average relative standard deviation (RSD) was 10.28 %, and the average recovery was 96.73 %, which indicated that the kit was highly sensitive and accurate. The icELISA kit remained usable for more than 6 months when stored at 4 °C. Therefore, the icELISA is a valuable tool for the rapid and sensitive detection of Gly m Bd 28K in food and feed products from soybeans.

Keywords Soybean allergen · Gly m Bd 28K · Monoclonal antibody · icELISA kit

Introduction

Gly m Bd 28K is a minor component (less than 0.5 % (w/w)) 7S globulin fraction of soybean seeds, but it is regarded as the most common soybean allergen together with Gly m Bd 30K and Gly m Bd 60K for infants and adults (Herman et al. 2003; Franke et al. 2008). Gly m Bd 28K can be recognized by serum antibodies in approximately 25 % of soybean-sensitive patients (Ogawa et al. 1991; Tsuji et al. 2001; Xiang et al. 2008). Approximately 1–2 % of adults and up to 5–7 % of children suffer from food allergies, a hypersensitive reaction of the human immune system (Mills and Breiteneder 2005; Martina et al. 2014). It is also reported that Gly m Bd 28K causes damage to intestinal morphology, contributes to disorders in the immune system, retards growth, and causes diarrhea (Liu et al. 2008). In consequence, a sensitive detection method for Gly m Bd 28K is needed to prevent soybean allergies from food and feed products.

Methods for the detection of food allergens could be based on proteins, such as the electrophoresis method (Rouquie et al. 2010), mass spectrometry (MS) (Cucu et al. 2012), and high-pressure liquid chromatography (HPLC) (Garcia and Marina 2006); detection can be based also on immunological methods that include enzyme-linked immunosorbent assay (ELISA) (Ma et al. 2010; You et al. 2008; L'hocine et al. 2007), EAST/RAST inhibition (Paschke et al. 2001), western blot (Janssen et al. 1986), and immunodiffusion (Iwabuchi and Yamauchi 1987). The detection of food allergens can also be based on DNA, such as real-time PCR (Torp et al. 2006) and multiplex PCR (Wang et al. 2010). In addition, many new technologies for detection the soybean allergens have been reported and include biosensors (Wang et al. 2011), microarrays (Kim et al. 2002), and others. These methods have high specificity and high sensitivity for the detection of allergens, although detection and quantification in the low ppb range are

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limited; we tend to require relatively inexpensive equipment that is suitable for high-throughput screening.

Up to now, commercial test kits have been developed to determine other antigenic proteins in soybeans such as P34, the α subunit of β -conglycinin, glycinin, and Gly m Bd 30K (Brandon et al. 1989; You et al. 2008; Liu et al. 2012). You et al. (2008) have reported that a competitive ELISA based on monoclonal antibody 6G₄ was established to determine β -conglycinin and shown an IC₅₀ value of 4.7 ng/mL with a detection limit of 2.0 ng/mL. But, we improved sensitivity for the detection of Gly m Bd 28K. ELISA methods for the detection of the Gly m Bd 28K have been reported in several studies (Noriko et al. 1998; Liu et al. 2012). Liu et al. (2012) synthesized the full-length complementary DNA (cDNA) sequence of Gly m Bd 28K chemically, and it was expressed in *Escherichia coli*; they then used a polyclonal antibody against the recombinant Gly m Bd 28K protein and established an iELISA. In general, when using polyclonal anti-sera, it takes a long time to purify the antigen to produce sufficient quantities of the antibody, the antigen that is obtained may still be contaminated by trace amounts of other proteins, and the anti-sera that is produced may not have strong specificity for the antigen (Poms et al. 2004).

It is currently known that there is no indirect competitive ELISA (icELISA) method to detect Gly m Bd 28K using a monoclonal antibody against the recombinant Gly m Bd 28K protein. In this study, the recombinant Gly m Bd 28K protein was expressed in *E. coli*, a mAb against recombinant Gly m Bd 28K was produced, and an icELISA kit based on the mAb was established and optimized.

Materials and Methods

Preparation of Recombinant Gly m Bd 28K Protein

Construction of a Prokaryotic Expression Vector

The soybean cDNA was generated by reverse transcription PCR from RNA isolated from soybean protein isolate. PCR was used to generate Gly m Bd 28K using the forward primer, 5'-GAATTCATGACAACAATGGCCTTCCATGATGATG-3', and the reverse primer, 5'-CTCGAGTTACTCCTCTTGGTCTTGATCATTTTCTT-3' (restriction sites in underlined). The conditions for PCR were 94 °C for 0.5 min, 57 °C for 0.5 min, and 72 °C for 2 min for 35 cycles, followed by a final extension step at 72 °C for 5 min. The PCR products were cloned into pGEM-T Easy (Promega) and transformed into *E. coli* JM109. Positive colonies were selected by blue/white selection on LB/Amp (100 μ g/mL) plates, and we grew 5 mL cultures overnight. The recombinant plasmid was extracted and the fragment identity was confirmed by DNA sequencing and then digested overnight with EcoRI/ XhoI at 37 °C. The Gly m Bd

28K cDNA fragment was extracted after electrophoresis and subcloned into the EcoRI and XhoI restriction sites of the pET28a expression vector and transformed into *E. coli* BL21 (DE3) competent cells to construct recombinant, expression plasmid pET28K.

Expression of Gly m Bd 28K in E. coli

The clone was streaked onto a LB agar plate that contained kanamycin (50 μ g/mL), and the plate was incubated at 37 °C for 15 h. A single colony was used to inoculate 10-mL LB medium containing kanamycin (50 μ g/mL) followed by its incubation at 37 °C in a shaking incubator. Five milliliters of the overnight culture was then used to inoculate a further 500 mL of LB-kanamycin in a 1000-mL culture flask and the culture growth continued in a 37 °C shaker for approximately 3 h. When the optical density (OD) reached 0.8 at 600 nm, isopropyl β -D-thiogalactoside (IPTG; 1.0 mM) was added. After 4 h of induction at 37 °C, cells were harvested by centrifugation at 5000 rpm for 30 min at 4 °C.

The cells were suspended in 50 mL of 0.01 M phosphate-buffered saline (PBS; pH 7.4). The cell suspension was sonicated on ice (99 $\times$ 3 s pulses with 10-s intervals) and centrifuged for 15 min at 12,000 rpm to separate the soluble and insoluble protein fractions. The supernatant was stored at 4 °C as the soluble fraction. The pellet was resuspended in 10 mL guanidine buffer (6 M guanidiniumhydrochloride, 50 mM Tris-HCl, 0.1 M NaCl, 10 % glycerol, and 10 mM DTT, pH 8.0). The lysis suspension was then centrifuged at 12,000 rpm for 10 min, and the supernatant fraction was stored at 4 °C as the insoluble fraction. The two protein fractions were analyzed by 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were detected in gels by Coomassie brilliant blue R-250 staining or transferred onto a nitrocellulose membrane for Western blot analysis using polyclonal antibody against Gly m Bd 28K.

Purification and Refolding of the Recombinant Gly m Bd 28K Protein

The fraction carrying the recombinant Gly m Bd 28K protein was purified by Ni-chelation chromatography. The volume of the resulting Gly m Bd 28K solution was adjusted to a protein concentration of 15 mg/mL (determined by BCA assay) and was used for subsequent refolding experiments. Eight microliters of the denatured protein solution was added dropwise in four pulses (2 mL each) to 500 mL of refolding buffer (0.4 M arginine, 50 mM Tris-HCl, 5 mM reduced glutathione, 0.5 mM oxidized glutathione, 0.5 mM PMSF, and 5 mM EDTA, pH 8.0 at 4 °C), with continuous stirring. The mixture was then stirred for 2 days until the concentration of free thiol groups was reduced to 1 mM by air oxidation, determined by Ellman's method (Ellman 1959). Any precipitate formed

during the refolding procedure was removed by centrifugation at 12,000 rpm for 30 min at 4 °C. The protein solution was then concentrated to 5 mL in a stirred cell concentrator (Millipore, Schwalbach, Germany) using a 5-kDa molecular weight cutoff (MWCO) membrane and rebuffered to 20 mM Tris-HCl pH 8.0. In the final purification step, the sample was loaded onto a Superdex-75 gel-filtration column equilibrated with 50 mM NaCl and 20 mM Tris-HCl, pH 8.0. Fractions that contained recombinant Gly m Bd 28K were pooled, rebuffered to 20 mM Tris-HCl pH 8.0, concentrated to 15 mg protein/mL by ultrafiltration (MWCO 5 kDa), and stored at 4 °C for preparation as immunogen antigen. The homogeneity of the protein was assessed by SDS-PAGE.

Production and Characterization of mAbs for Gly m Bd 28K

Six BALB/c female mice (7–8 weeks old, purchased from the medical college of Zhengzhou University, China) were immunized with the recombinant Gly m Bd 28K by subcutaneous injections at multiple points. The first dose consisted of 40 µg of immunogen as an emulsion of PBS (8.0 g NaCl, 0.2 g KH₂PO₄, 2.13 g Na₂HPO₄, and 0.2 g KCl per liter, pH 7.4) and Freund's complete adjuvant (Sigma Chemical Co., St. Louis, MO, USA). Three subsequent injections were given at 3-week intervals with the same dosage of immunogen emulsified in Freund's incomplete adjuvant. Ten days after the fourth injection, serum was collected, and based on the indirect and the block ELISA determination of the antibody titers, mice that showed the highest titer and sensitivity were selected to be spleen donors for hybridoma production.

The mice received a final soluble intraperitoneal (i.p.) injection of 40 µg of conjugate in PBS. Three to 4 days later, the splenocytes were isolated and fused with myeloma cells at a ratio of 10:1 using PEG 4000 (Hyclone, GE Healthcare Life Sciences, USA). The fused cells (hybridomas) were distributed in 96-well culture plates (Nunc-Immuno Plate MaxiSorp, Denmark) that were supplemented with HAT medium containing 15 % FBS (Sigma Chemical Co., St. Louis, MO, USA) with peritoneal macrophages from young BALB/c mice feeder cells. At 10–14 days after fusion, supernatants of hybridoma colonies were screened using a combination of non-competitive and competitive ELISA for the presence of Gly m Bd 28K mAbs. Selected wells were subcloned by limiting dilution, colonies of interest were frozen in culture medium that contained 10 % dimethyl sulfoxide, and colonies were cryopreserved in liquid nitrogen and then defrosted three times to select the stable antibody-producing clones.

A mature female BALB/c mouse was injected intraperitoneally (i.p.) with 0.5 mL paraffin 10 days before receiving an i.p. injection of the hybridoma cells that were suspended in

RPMI 1640 medium (Gibco, Thermo Fisher Scientific, USA). Ascites fluid was collected 10 days after the injection and stored at −20 °C until use. Purification of mAb was achieved by saturated ammonium sulfate precipitation. Measurement of mAb affinity (K_a) was carried out according to Beatty et al. (1987). The class and subclass of the isotypes of the purified antibody were determined by using a mouse mAb isotyping kit (Sigma Chemical Co., St. Louis, MO, USA).

Development of the Gly m Bd 28K icELISA Kit

The Working Concentration of Recombinant Gly m Bd 28K and Gly m Bd 28K mAb

It is well known that working concentrations of coating antigen and antibody are crucial for determining the sensitivity of ELISA methods. Checkerboard tests were performed to determine the optimal dilution of recombinant Gly m Bd 28K and Gly m Bd 28K mAb, which resulted in the following optimized protocol. The relative parameters for icELISA are listed in Table 2, the initial concentration of recombinant Gly m Bd 28K was 0.1 mg/mL, and the initial concentration of Gly m Bd 28K mAb was 0.5 mg/mL.

Reaction Times of Gly m Bd 28K mAb

Three factors will affect the result: Gly m Bd 28K mAb incubation time, goat anti-mouse IgG-HRP (GaMIgG-HRP) incubation time, and enzyme developing time. The microplates were coated at the optimal concentration of recombinant Gly m Bd 28K at 37 °C, but all other operations were conducted at 25 °C and avoided light. The optimal times for the adsorption of the antibodies and for substrate development were determined by conducting a primary orthogonal test (Table 1).

Assembly of the icELISA Kit

The kit includes a 96-well microtiter plate (one piece) coated with recombinant Gly m Bd 28K and blocked with blocking buffer in advance, the working concentration of Gly m Bd 28K mAb and GaMIgG-HRP solutions (0.2 mL,

Table 1 Orthogonal design header of the best working time

Level	Factor (min)		
	Gly m Bd 28K mAb (α)	GaMIgG-HRP (β)	Substrate (γ)
1	15	20	10
2	20	35	15
3	25	50	20

respectively), standard solutions of recombinant Gly m Bd 28K (32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0 $\mu\text{g/L}$), substrate solution A, substrate solution B, stopping solution (8 mL, respectively), washing buffer (20 mL), and PBS. A protocol sheet that contains operational steps and a sample standard curve are included. Kits are sealed in a plastic bag in the presence of desiccant gel and stored at 4 °C.

Establishment of Standard Curve

The recombinant Gly m Bd 28K standard sample was prepared by diluting the initial solution prepared in PBS (pH 7.4) to give the working standards (0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 $\mu\text{g/L}$). These were tested using the developed icELISA kit with three replicates per concentration. According to the inhibition titer that was determined by blocking ELISA, the standard curves were obtained by normalized absorbance values ($B/B_0\%$), where B_0 is the absorbance at zero concentration of recombinant Gly m Bd 28K and B is the absorbance of the sample with recombinant Gly m Bd 28K.

Characterization of icELISA Kit

Sensitivity According to Hayashi et al. (2005), the sensitivity of the kit was measured by the formula $B/B_0\% = 83.3\%$. The concentration of recombinant Gly m Bd 28K could be calculated through the standard curve regression equation. The results are detection limit and sensitivity.

Recovery According to the affix recovery test, the recoveries are acquired. Briefly, the assay was evaluated by performing triplicate measurements of the recombinant Gly m Bd 28K samples diluted to 0.5, 1.0, and 2.0 $\mu\text{g/mL}$. The recovery was determined using the standard curve of the kit.

Stability The kits were kept in a refrigerator at 4 °C, and the performance was determined at a fixed period; at each stage, the IC_{50} was recorded.

Detection of Gly m Bd 28K

Various soybean products from soybean seeds, soybean protein isolate, soybean meal, tofu, soy milk, soy sauce, natto, preserved bean curd, and lobster sauce were diluted in Tris-HCl buffer. The Gly m Bd 28K content from these soybean products was analyzed by the icELISA developed above. Each sample was assayed six times per plate, and we used a triplicate array of plates.

Results and Discussion

Recombinant Gly m Bd 28K Production and Characterization

Expression and Characterization of Recombinant Gly m Bd 28K in *E. coli*

After IPTG induction, the *E. coli* BL21(DE3) that harbored pET28K plasmid expressed a recombinant protein with a molecular weight around 29.1 kDa compared to the control cells that contained pET-28a vector. The recombinant Gly m Bd 28K could only be detected in the pellet fraction after sonication and centrifugation, which indicated that it existed mainly as insoluble aggregates (Fig. 1a). Western blot analysis revealed that recombinant Gly m Bd 28K can bind polyclonal antibody against Gly m Bd 28K (Fig. 1b).

Purification and Refolding of Recombinant Gly m Bd 28K

The protein overexpressed in *E. coli*, as is commonly the case, formed insoluble inclusion bodies. After four washes, inclusion bodies were obtained. The inclusion bodies were dissolved in 6 M guanidine buffer and purified by Ni-chelation chromatography. The denatured protein was diluted rapidly in a large volume of the refolding buffer at 4 °C and allowed to equilibrate for 2 days. After concentration, the functional,

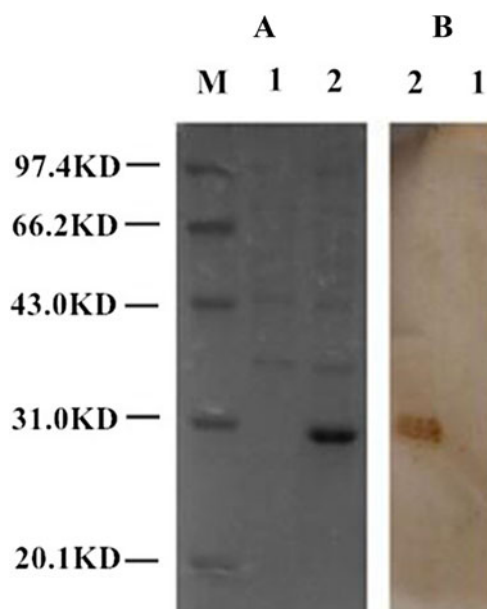


Fig. 1 Analysis of Gly m Bd 28K expression in *E. coli* BL21(DE3). **a** Coomassie blue-stained gel after SDS-PAGE. **b** Western blot analysis. Lane M, protein molecular weight markers. Lane 1, soluble fraction of pET28K/BL21(DE3) cell lysate. Lane 2, insoluble fraction of pET28K/BL21(DE3) cell lysate

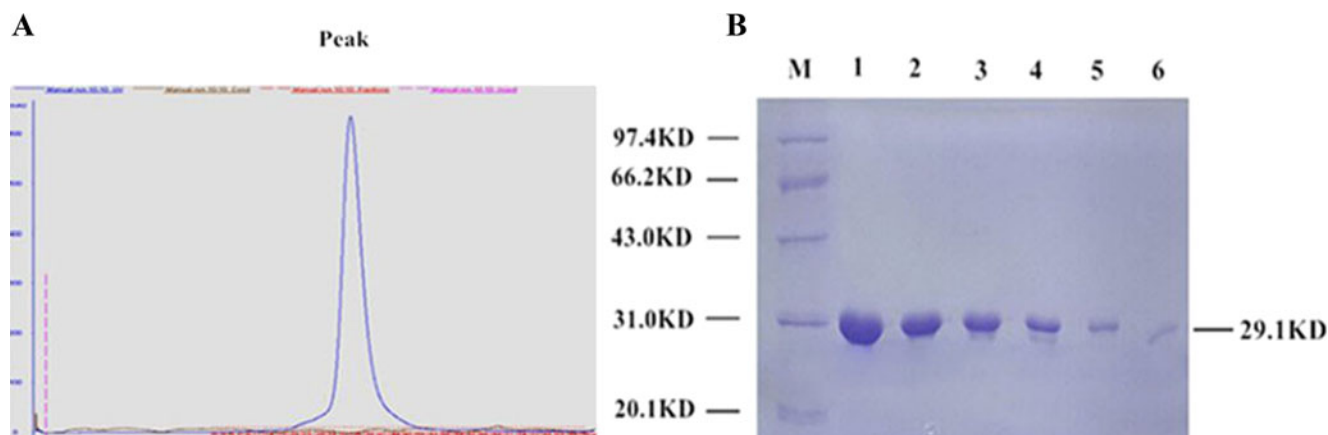


Fig. 2 **a** Sephadex G-75 gel-filtration column analysis of recombinant Gly m Bd 28K inclusion body after refolding by rapid dilution peak monomer protein; this fraction was pooled. **b** SDS-PAGE of fractions from the peak stained by Coomassie Brilliant Blue R250

refolded, recombinant Gly m Bd 28K protein was recovered by using a superdex-75 column (Fig. 2a). The purity was up to 90 %, as determined by SDS-PAGE (Fig. 2b).

Production and Characterization of Monoclonal Antibodies

After the cell fusion procedure, growing hybridoma cell clones could be observed in many of the wells of seeded 96-well plates. The fusion rate of the mouse spleen cells with myeloma cells was approximately 80 %, and the positive well rate was 20 %. Finally, a total of three high-response hybridomas against Gly m Bd 28K were obtained and the mAbs from ascites fluid were purified and characterized. Using a mouse mAb isotyping kit, all three antibodies were of the IgG1 isotype with a k light chain. The affinity constants for each of the three selected hybridomas were measured by ELISA using serial dilutions of both the coated antigen and mAb ($K_{\text{aff}} = (n-1)/2(n[\text{Ab}']t - [\text{Ab}]t)$, $n = [\text{Ag}]t/[\text{Ag}']t$, the K_{aff} s of 3H6 mAb, 2D9 mAb, and 3F5 mAb were 3.24×10^9 , 2.67×10^9 , and 4.02×10^{10} L/mol, respectively). Based on Goding (1983), the three hybridomas produced

high-affinity mAbs (between 10^7 and 10^{12} L/mol). The antibody was highly specific for Gly m Bd 28K, and the most sensitive hybridoma, which we named 3F5, showed an IC_{50} value of 3.49 $\mu\text{g/L}$.

Development of the icELISA Kit

Optimum Concentration of Coating Antigen

From the checkerboard assays for the direct format, we selected the optimal condition of the icELISA in which the OD value was approximately 1.0, so the optimal concentration for recombinant Gly m Bd 28K was 1 $\mu\text{g/mL}$ and 1:64,000 for the mAb (Table 2).

Optimal Working Time

Our results for the orthogonal testing indicated that the working time for 3F5 mAb was theoretically 15 min, the working time for GaMIgG-HRP was theoretically 50 min, and the enzyme development time was theoretically 10 min (Table 3).

Table 2 The result of checkerboard

Dilution of mAb	Concentration of recombinant Gly m Bd 28K ($\mu\text{g/mL}$)					
	0.125	0.25	0.50	1.00	2.00	4.00
1:16,000	1.471	2.060	1.875	1.769	2.195	2.158
1:32,000	1.270	1.830	1.759	1.403	2.064	2.051
1:64,000	1.138	1.732	1.423	1.133	1.987	1.856
1:128,000	0.970	1.363	1.167	0.983	1.356	1.593
1:256,000	0.798	1.012	0.934	0.887	0.794	1.008
1:512,000	0.720	0.510	0.779	0.659	0.507	0.646
1:1,024,000	0.424	0.302	0.281	0.400	0.373	0.300
Negative	0.145	0.204	0.213	0.199	0.201	0.176
Blank	0.063	0.068	0.057	0.065	0.059	0.067

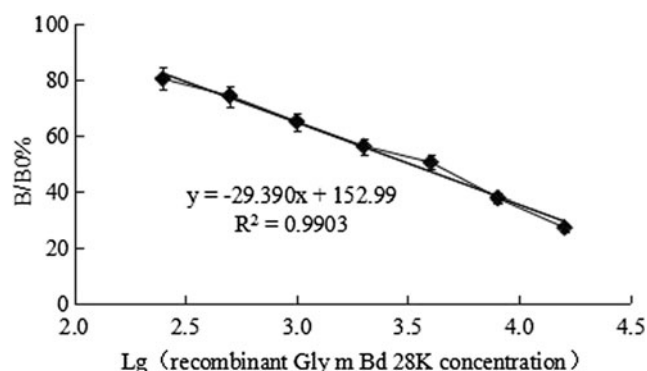
Table 3 Results of orthogonal tests

Numbers	A	B	C	P/N
1	1	1	1	2.062
2	1	2	2	3.507
3	1	3	3	3.075
4	2	1	2	3.002
5	2	2	3	4.014
6	2	3	1	3.903
7	3	1	3	3.106
8	3	2	1	2.907
9	3	3	2	2.741
K ₁	2.723	2.957	2.939	
K ₂	3.476	3.083	3.505	
K ₃	3.240	3.398	2.995	
R	0.753	0.441	0.566	
Best combination	A ₂	B ₃	C ₂	
Major factor		A > C > B		

However, it is worth mentioning that each kit has its own optimum workflow, so the workflows we obtained will not apply to all kits.

Test Procedure

We removed the required number of coated microplates and washed them with washing buffer three times. Then, we added 50 µL/well of analyte and 50 µL/well of Gly m Bd 28K mAb and incubated the plates for 15 min at 37 °C. The solution was then discarded, and plates were washed five times with washing buffer. Then, GaMIgG-HRP (1:1000, 50 µL/well) was added, followed by incubation for 50 min at 37 °C. The final washing procedure was followed by color development, which was initiated by the addition of 50 µL/well of substrate solutions A and B (1:1, v/v) that were freshly prepared. After incubating at room temperature for 10 min, the reaction was stopped using 2 mol/L H₂SO₄. The absorbance values were

**Fig. 3** The standard curve of icELISA kit**Table 4** Recovery analysis of the icELISA kits

Gly m Bd 28K (µg/mL)	Mean (µg/mL) ^a	SD	Recovery (%)	RSD (%)
0.5	0.48	0.051	96.00	10.63
1.0	1.01	0.11	101.00	10.78
2.5	2.33	0.22	93.20	9.44

^a The data represent the means of three replicate subsamples

measured at 450 nm, and the inhibition ratio ($B/B_0\%$) was calculated. Afterward, the standard curve can be established, and according to the regression equation from the standard curve, the amount of Gly m Bd 28K in the sample can be calculated.

Standard Curve of the icELISA Kit

The competitive curve obtained with the icELISA allowed the detection of recombinant Gly m Bd 28K concentrations from 0 to 32 µg/L. The x -axis is expressed as a logarithmic concentration. The y -axis is expressed as normalized absorbance values ($B/B_0\%$), where B_0 is the absorbance at zero concentration of recombinant Gly m Bd 28K and B is the absorbance of the sample with recombinant Gly m Bd 28K. Data represent the means of three determinations (Fig. 3). According to the regression equation, IC₅₀ was determined at 3.19 µg/L.

Characterization of the icELISA Kit

According to the formula $B/B_0\% = 83.3\%$, the concentration of recombinant Gly m Bd 28K was calculated, and the result was 0.235 µg/L, that is, for this kit detection limit and sensitivity. The recoveries of recombinant Gly m Bd 28K are shown in Table 4, and the data represent the means of three determinations. The average recoveries of Gly m Bd 28K were 96.73 % (recovery ranges were between 93.20 and 101.00 %), and the average relative standard deviation (RSD) was 10.28 % (RSD ranges were between 9.44 and 10.78 %). The kits were always stored at 4 °C in a refrigerator

Table 5 Validity of the kit

Storage time (days)	IC ₅₀ (µg/L)	R ²	A _{450nm}
1	3.19	0.9903	1.112
30	3.44	0.9846	1.011
60	3.14	0.9817	0.982
90	2.97	0.9907	0.904
120	3.21	0.9745	0.837
150	3.05	0.9687	0.783
180	3.20	0.9768	0.695

Table 6 Contents of Gly m Bd 28K in Soybean Products

Product ^a	Gly m Bd 28K content (μg/g)	CV (%)
Soybean seeds	4.85 ± 0.05	10.97
Soybean protein isolate	14.86 ± 0.23	9.12
Soybean meal	2.06 ± 0.07	10.23
Tofu	0.41 ± 0.12	9.18
Soy milk	0.21 ± 0.02	11.40
Soy sauce	— ^b	—
Natto	— ^b	—
Sufu	— ^b	—
Lobster sauce	— ^b	—

^a The data represent the means of six replicate subsamples

^b Not detected

and were taken out to determine the IC₅₀ value after 1, 30, 60, 90, 120, 150, and 180 days. With the increase of storage time, the value of A_{450nm} gradually becomes small, but the IC₅₀ value and correlation coefficient (R^2) had little change; the results showed that the kit has a good “shelf life” (Table 5).

Detection of Gly m Bd 28K

The iELISA was used to determine the Gly m Bd 28K content of soybean seeds, soybean protein isolate, soybean meal, tofu, soy milk, soy sauce, natto, sufu, and lobster sauce. Of the products tested, soybean seeds, soybean protein isolate, soybean meal, and tofu were shown to contain high concentrations of the Gly m Bd 28K (Table 6). The fact that the allergen occurs at high concentrations in these products suggests that most of the allergens from soybeans remains unchanged during processing. Soy milk contained less allergens than the above mentioned soybean products, although it is produced by soybean seeds, which indicated that the epitopes on the allergen against the mAbs might be modified during the polymerization of the proteins in soybean seeds and that the mAbs could not bind to the modified epitopes. Soy sauce, natto, preserved bean curd, and lobster sauce from fermented soybean products contained no allergens. This result suggests that the allergens in the product had been hydrolyzed rapidly by the proteinases of microorganisms during fermentation.

Compared with previous studies (Noriko et al. 1998; Liu et al. 2012), the development of an iELISA kit for the detection of soybean allergenic protein Gly m Bd 28K has higher sensitivity and specificity and could be applied to analyze real samples. Thus, it meets the technical requirements for quality control and food safety related to soybeans and provides a valuable tool for the rapid and sensitive detection of Gly m Bd 28K in soybeans and food products derived from soybeans.

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Compliance with Ethical Standards

Conflict of Interest Jun Xi declares she has no conflict of interest. Qiaoqiao Shi declares she has no conflict of interest. This article does not contain any studies with human subjects.

Ethical Approval and Animal Rights The studies with animals were performed following all institutional and national guidelines for the care and use of laboratory animals.

Informed Consent Not applicable.

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