Published in final edited form as:

Anal Biochem. 2018 October 15; 559: 55–61. doi:10.1016/j.ab.2018.08.012.

Peptide ligand-based ELISA reagents for antibody detection

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Abstract

Detection of specific antibodies has numerous research, therapeutic and diagnostic applications. Short peptide ligands that bind specifically to antibodies with continuous epitopes can be derived from epitope mapping experiments. Short peptide ligands (mimotopes) specific to antibodies with discontinuous epitopes can be identified by screening complex peptide libraries. In an effort to enhance practical utility of such peptide ligands, we describe here a simple approach to turn such target antibody-specific peptide ligands into specific ELISA detection reagents. We show that a simple addition of biotinylated peptide ligands to commonly available horseradish peroxidase (HRP)-labeled streptavidin (or HRP-anti-biotin antibody), or digoxigeninlabeled peptides to HRP-anti-digoxigenin antibody detection reagents transformed these generic detection reagents into sensitive target antibody-specific reagents. ELISA assays performed using these reagents exhibited excellent analytical properties indicating their practical utility for antibody detection. One generic detection reagent can be readily transformed into many different specific ELISA reagents by a simple mix and match design using an appropriate target-specific peptide ligand. Simplicity of preparation of these ELISA reagents for detecting antibodies should facilitate their practical applications.

Keywords

Antibody; detection; ELISA; peptide

1. Introduction

Assays for detecting antibodies have numerous applications in research and clinical diagnostics. Infections induce an immune system response allowing diagnosis of the disease with assays detecting host antibodies produced in response to the infection [1–4]. Many diseases have an autoimmune component where disease-specific circulating autoantibodies are produced providing opportunities for using such autoantibodies as disease biomarkers. Circulating autoantibodies were found in autoimmune diseases (for example, in Type 1

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diabetes [5, 6] or in lupus [7]), cancer [8–10], cardiac dysfunction [11] and neurologic diseases [12]. Increased use of protein therapeutics produces a need to detect and quantitate the neutralizing circulating antibodies that a patient might produce against these therapeutics [13]. There is thus a continuing interest in developing and improving assays for antibody detection.

Antibodies are most often detected using ELISA assays where the target antibody is captured by an immobilized antigen and detected by a secondary detection antibody even though this assay format often has significant performance issues [14]. The use of antigens for antibody capture in ELISA assays, while conceptually obvious, has potential drawbacks. Purified antigens may be difficult to obtain, may be unstable and may undergo conformational changes upon immobilization on solid support that might negatively affect their recognition by their cognate antibodies.

Short peptides that bind specifically to target antibodies are valuable ligands with utility in antibody assays. Short peptides became important reagents in epitope mapping when the technology to synthesize large numbers of peptides on solid support was developed in 1980's [15]. These technologies were further refined to produce peptide arrays that can display thousands of peptides on a solid support [16]. Relative chemical stability of short peptides and the relative ease by which they can be synthesized and manipulated makes them also desirable ligands in development of novel antibody assays. For example, we have described peptide-based homogenous and heterogeneous assays that utilize oligonucleotide hybridization turned on by binding of oligonucleotide-peptide conjugates to the antibody to generate a convenient signal reporting the presence of the target antibody [17, 18]. Short peptide ligands for antibodies recognizing simple linear epitopes of protein antigens can be designed using data from epitope mapping experiments. Many (if not a majority) of biomedically relevant antibodies likely recognize more complex discontinuous epitopes where residues from different regions of the protein are brought to spatial proximity by folding of the protein to form a functional antibody epitope [19, 20]. Short peptide ligands for such antibodies derived from epitope mapping would typically represent only a partial epitope and thus would likely have low affinity for the antibody [20]. However, it is well documented that short peptide ligands (mimotopes) mimicking the original discontinuous epitope could be often identified [21–23]. Mimotopes are most often obtained by screening of combinatorial peptide libraries for binding activity to a desired target [23]. We have demonstrated that ribosome display enhanced by Next Generation Sequencing (NGS) is particularly efficient in identifying mimotopes for antibodies with discontinuous epitopes or epitopes containing posttranslational modifications [24]. Furthermore, peptide mimotopes were also developed for antibodies recognizing antigens other than proteins, such as, for example, DNA [25] or even small haptens such as vitamin D [24]. We conclude thus that for a majority of antibodies (including antibodies against non-protein antigens) where an interest in developing of assays to detect them might exist, it should be possible to obtain short peptide ligands that could selectively bind the target antibody.

In a search for new utilities for such antibody-specific short peptides, we describe here a simple idea to use such peptides to transform commonly available non-specific detection reagents into highly specific ELISA detection reagents. We show that by simple mixing of

nonspecific detection reagent (for example, HRP-labeled streptavidin) with appropriately functionalized antibody-specific peptide (for example, biotinylated peptide) an ELISA detection reagent can be readily prepared and used for sensitive and specific measurement of the target antibody.

2. Materials and Methods

2.1. Materials.

Antibodies were obtained from the following sources: anti-digoxigenin HRP-conjugated affinity-purified chicken antibody was from Gallus Immunotech (Fergus, ON, Canada), goat polyclonal anti-human Troponin I antibodies (specific to peptide 1–15 and to peptide 16–26) were from Biospecific (Emeryville, CA), anti-biotin HRP-conjugated monoclonal mouse antibody, anti-Goat IgG affinity purified bovine antibodies and HRP-conjugated streptavidin were from Jackson ImmunoResearch Laboratories (West Grove, PA), mouse monoclonal anti-human phosphotroponin was from Abcam (Cambridge, MA) and anti-p53 (pAb1620) was from Calbiochem (La Jolla, CA). Pierce™ protein G- coated clear 8-well strips, Pierce™ neutravidin-coated clear 8-well strips, NUNC flat-bottom MaxiSorp polystyrene 96-well microplates, 1-Step™ Ultra TMB-ELISA and SuperBlock^R blocking buffer were purchased from ThermoFisher Scientific. Human serum was obtained from Sigma-Aldrich (St. Louis, MO).

2.2. Peptide synthesis and modification.

The following derivatives of anti-Troponin I antibody (anti-human Troponin I specific to peptide 1–15) antigen peptide (MADGSSDAAREPRPA) were prepared: P1, AADGSSDAAREPRPA; P2, VADGSSAAARDPPPA; P3, TADGASAAAAREPGPA; P1 scrambled, RMEPSDADGARSPAA. P1 peptide corresponds the antigen sequence with a single amino acid replacement of N terminal M with A. P2 and P3 peptides correspond to mutant antigen peptide sequences designed using alanine scanning binding data (Fig. S1, Supplementary Information) to exhibit progressively lower binding affinity to the anti-Troponin I antibody. P1 scrambled is a negative control (scrambled wt antigen sequence). The peptides were synthesized using F-moc chemistry on a PS3 automated peptide synthesizer (Protein Technologies, Inc., Tucson, AZ).

The N-termini of the peptides were modified with biotin by reacting de-protected N-terminal amino group of the peptides attached to the resin with EZ-Link NHS-PEG₄-biotin (Pierce, Rockford, IL). To remove the N-terminal Fmoc, the resin with synthesized peptide was treated for 5 min with DMF:piperidine (4:1 volume). The resin was washed with DMF and resuspended again in DMF:piperidine for 5 min followed by washes with DMF and methanol. ~8.5 micromoles of the resin were suspended in 250 µl of DMF containing 10 µl of N,N-diisopropylethylamide and were mixed with 250 µl of DMF containing 5 mg of NHS-PEG₄-biotin. Modification reaction was allowed to continue overnight at room temperature. 500 µl of DMF were added to the reaction mix and the resin suspension was transferred to 1.5 ml syringe filter. The resin was washed with 1 ml of DMF followed by 4 washed with 1 ml of methanol and was dried. Labeled peptides were cleaved from the resin and deprotected by suspending the resin in 500 µl of 94:2.5:2.5:1 TFA:water:EDT:TIPS mix,

incubating the mix on ice for 1.5 min followed by 1.5 hours at room temperature. The supernatant was collected to a tube containing 30 ml of ice-cold diethyl ether. Precipitated peptides were collected by centrifugation at 3000g for 5 min, the pellets were washed with 5 ml of cold diethyl ether and were dried in Speed Vac. Biotin-labeled peptides were purified by HPLC on a C18 4.6×250 mm column (Vydac) using 0–50% acetonitrile gradient. Peaks containing labeled peptides were dried in Speed Vac, dissolved in 80:20 water:DMF and stored at –20 deg. The concentration of stock peptide solutions was determined by measuring the biotin label concentration with a fluorescent assay for biotin [26].

Modification of the N-termini of the peptides with digoxigenin was performed by reacting deprotected and cleaved peptides in solution with digoxigenin NHS ester (Enzo Life Sciences, Farmingdale, NY). ~ 1.1 mg of crude peptides were first purified by HPLC on a C18 4.6×250 mm column (Vydac) using 0–50% acetonitrile gradient. Purified peptides were dissolved in 70 µl of DMF containing 3 µl of triethylamine and 20 µl of DMF containing 0.2 mg of digoxigenin NHS ester were added to start the reaction. Reaction mix was incubated overnight at room temperature. Reaction mixtures were diluted with water (100 µl) and modified peptides were purified by HPLC on a C18 4.6×250 mm column (Vydac) using 0–50% acetonitrile gradient. Peaks containing labeled peptides were dried in Speed Vac, dissolved in 80:20 water:DMF and stored at –20 deg.

2.3 ELISA assays with anti-Troponin I antibody capture on protein G coated plates and detection by peptide-based reagents.

Wells were washed with 200 µl of PBS buffer containing 0.05% Tween 20 and 1 mg/ml of BSA (PBST-BSA). Samples with desired amounts of antibodies in 100 µl PBST-BSA or 1:4 mix of human serum and PBST-BSA were placed in protein G-coated wells of a microplate. The samples were incubated for 2 hrs at room temperature and the wells were washed with 3× 200 µl of PBS buffer + 0.05% Tween 20 (PBST). The detection reagent (a mix of the biotin or digoxigenin-labeled peptide and matching HRP-conjugated detection reagent in PBST-BSA) was then added (100 µl). Detection reagents were prepared freshly by mixing appropriate dilutions of streptavidin-HRP (or other HRP-conjugated detection reagents) with the peptide functionalized with a moiety (biotin or digoxigenin) matching specificity of HRP-labeled reagent. After 1 hr incubation at room temperature, wells were washed with 3× 200 µl of PBST and 100 µl of TMB substrate was added to each well and after 2–6 min the reaction was stopped 100 µl of 2 M sulfuric acid. Absorbance at 450 nm was read on a SpectraFluor Plus microplate plate reader (Tecan).

2.4. ELISA assays with anti-Troponin I antibody capture on microplates coated with species-specific anti-IgG antibody detection by peptide-based reagents.

The wells were washed with 1×200 µl of coating buffer (0.2 M sodium carbonate/bicarbonate buffer, pH 9.2) and 100 µl of 100 nM anti-goat IgG antibody in coating buffer was loaded into each well. The plates were incubated at 4 deg overnight and were washed with 3× 200 µl of PBST followed by blocking with 3× 300 µl of SuperBlock^R blocking buffer. Samples were loaded by adding to the wells desired amounts of antibodies in 100 µl PBST-BSA or 1:4 mix of human serum and PBST-BSA. Detection reagents and procedures were the same as described for protein G coated plates.

2.5. ELISA assays with anti-Troponin I antibody capture by non-specific adsorption on MaxiSorp microplates and detection by peptide-based reagents.

The wells were washed with 1×200 µl of coating buffer. Samples were loaded by adding to the wells desired amounts of antibodies in 100 µl coating buffer. After 2 hrs. incubation at room temperature, the wells were washed with 3×200 µl of PBST followed by blocking with 3× 300 µl of SuperBlock^R blocking buffer. Detection reagents and procedures were the same as described for protein G coated plates.

2.6. ELISA assays with anti-Troponin I antibody capture enabled by the peptides and detection by species-specific anti-IgG HRP-conjugated antibody.

The wells of PierceTM neutravidin-coated clear 8-well strips were washed with 3×200 µl of PBSTBSA. The wells were functionalized with the peptides by loading desired concentration of biotinylated peptide in 200 µl PBST-BSA to the wells. The peptides were allowed to bind neutravidin for 1 hr. at room temperature. The wells were washed with 4×200 µl of PBST-BSA and 100 µl of antibody samples in dilution buffer were loaded to the wells. After 1.5 hrs. incubation at room temperature, the wells were washed with 4×200 µl PBST-BSA. Anti-goat IgG antibody (HRP-labeled) (100 µl of 1:40000 dilution in PBST-BSA) was then added to each well. After 1 hr incubation at room temperature, wells were washed with 3× 200 µl of PBST and 100 µl of TMB substrate was added to each well. Detection and absorbance readout were the same as in case of protein G coated plates.

All ELISA assays were done in triplicate and standard deviation of the signals were calculated. Limits of detection (LOD's) were calculated as a ratio of 3xstandard deviation of ELISA signals and the slope of linear regression of ELISA signal versus antibody concentration.

3. Results and Discussion

3.1 Design of peptide-based ELISA reagents

Fig. 1A illustrates design of peptide-based detection reagent. Target antibodies are immobilized in wells of the microplate using standard procedures such as, non-specific adsorption on polystyrene plates, protein G-mediated or species-specific anti-IgG antibody mediated immobilization. We hypothesized that simple mixing of antibody-specific biotinylated peptide with non-specific HRP-streptavidin detection reagent should produce an effective antibody-specific detection reagent. The specificity of resulting reagents would be determined by the identity of the peptide used to functionalize HRP-streptavidin and could be thus easily manipulated to produce reagents for a wide range of target antibodies.

We used a model target antibody (anti-human Troponin 1) and a set of peptides that bind the antibody with different affinities to test and validate the design illustrated in Fig. 1. Our model antibody recognizes N-terminal peptide of the troponin. We performed alanine scanning experiment (Supplementary Fig. S1) to identify which residues of the antigen peptide were important for binding to the antibody. Based on these data, we designed 3 sequence variants of the antigen peptide with different binding affinities (K_d 's of 5, 70, and 280 nM for P1, P2 and P3 peptides, respectively) for the antibody (Supplementary Fig. S2).

3.2. Peptide-based ELISA detection reagents

In the first set of the experiments (Fig. 2) we employed capture of anti-Troponin I antibody on protein G covered microplates and tested specificity of the detection reagent produced by mixing HRP-streptavidin with biotinylated detection peptides. Positive ELISA signals were observed with all three peptides whereas no signal was observed with scrambled P1 peptide (Fig. 2A). As expected, the peptide (P1) with the highest affinity for the antibody produced the highest signal (Fig. 2A). However, peptide with the lowest binding affinity (P3) produced similar signal compared to P2 (peptide with moderate binding affinity) (Fig. 2A) indicating that equilibrium binding affinity is not a perfect predictor of performance of peptide-based detection reagent. Since ELISA assays involve extensive washing of the wells after incubation with detection reagent, dissociation kinetics of the peptide will likely play an important role as well.

Experiments in Fig. 2 were performed using detection reagents obtained by mixing biotinylated peptides with HRP-streptavidin at 3:1 molar ratio. This ratio was selected based on an experiment where we examined the dependence of ELISA signal on peptide to HRP-streptavidin ratio (Supplementary Fig. S3). We observed that the ELISA signal increased with the increase of the ratio up to the ratio of ~2:1. Ratios higher than 2:1 did not produce a significant increase of the signal. We selected the ratio of 3:1 for subsequent experiments.

We compared ELISA signals obtained for the anti-Troponin I antibody and 3 control antibodies (Fig. 2B) to further examine the specificity of the peptide:HRP-streptavidin reagent. No significant cross reactivity was observed (Fig. 2B) at the same or 10x higher amount of the control antibodies (only in the case of antibody to a different epitope of human Troponin I (anti-Troponin I aa 16–26, Fig. 2B), a small ELISA signal at 10x amount of control antibody was observed). Fig. 3A illustrates anti-Troponin I antibody concentration dependence of ELISA signals. The signals were reproducible ($CV < 0.15$) and a linear relationship between the signal and antibody concentration was observed in the range of antibody concentrations tested (0–1.6 ng/well). The limit of detection (LOD) estimated from the data in Fig. 3A was 62 pg/well. Based on data in Fig. 2&3A we concluded that a simple mixing of biotinylated antibody-specific peptide with HRP-streptavidin produces a functional target antibody-specific ELISA detection reagent. ELISA assay enabled by such reagent exhibited excellent analytical performance.

Biotinylated peptides, in addition to HRP-streptavidin, could be also paired with different biotin-specific detection reagents. For example, we tested HRP-anti-biotin antibody paired with biotinylated P1 and observed that it also produced a functional ELISA detection reagent (data not shown). However, compared to HRP-streptavidin, HRP-anti-biotin reagent exhibited lower ELISA signals at the same anti-Troponin I antibody concentrations and higher LOD (not shown). Peptide-based detection reagents are not limited to using just biotin as a tag bridging peptide with non-specific detection reagent. To test this, we labeled P1 peptide with digoxigenin and tested if digoxigenin-P1 paired with anti-digoxigenin HRP-antibody would also produce a functional ELISA detection reagent (Fig. 3B). In this assay we used chicken anti-digoxigenin HRP-antibody since chicken antibodies do not bind to protein G allowing the use of protein G coated microplate for the capture of the target antibody. ELISA assay with this alternative reagent also exhibited excellent analytical

characteristics with LOD of 300 pg/well (Fig. 3B) that was somewhat worse compared to biotin-P1 paired with HRP-streptavidin (Fig. 3A).

One of the main applications of ELISA assays for detecting antibodies is to measure the amounts of antibody in serum for diagnostic or research purposes. We tested the utility of peptide-based ELISA reagents for the detection of antibodies in serum using serum samples spiked with different amounts of anti-Troponin I antibody (Fig. 4A). ELISA signals measured were proportional to the amount of anti-Troponin I antibody spiked into the serum but compared to the assay in a buffer (Fig. 3A) the reproducibility of the signal at each antibody concentration was worse and LOD was ~100-fold higher (6.6 ng/well; Fig. 4A). Increased LOD in serum for an assay format used in Fig. 4A was expected. IgG in serum present at high concentrations could bind non-specifically to P1 reducing its specific binding to the anti-Troponin I antibody. Also, IgG in serum will bind to protein G decreasing the efficiency of capture of anti-Troponin I antibodies on protein G-covered microplate wells. Nevertheless, the observed LOD (6.6 ng/well) corresponds to ~260 ng/ml of antibody in serum which should be sufficient for most practical applications of the assay. For example, the levels of cancer specific autoantibodies in serum (positive cut-off values reported for seven autoantibodies in breast cancer were from 280 to 1,200 ng/ml [27]) would be within the sensitivity of the assay in Fig. 4B. The levels of antibodies produced in response to infections would be typically much higher (for example, more than 10-fold [28]).

Experiments in Fig. 2 demonstrated that peptides with very different binding affinities for antibody produced functional ELISA detection reagents when paired with HRP-streptavidin. We tested analytical performance of the P3-HRP-streptavidin detection reagent (Fig. 4B) to determine how its ~50-fold lower affinity for the anti-Troponin I antibody (compared to P1 peptide) affected performance of the detection reagent. The observed LOD was ~30-fold higher (1.8ng/well; Fig. 4B) which was roughly proportional to antibody binding affinity difference between P1 and P3. Also, the dependence of ELISA signals on anti-Troponin I antibody concentration was non-linear. The data in Fig. 4B demonstrate, as expected, that affinity of the peptide for the antibody plays an important role in defining analytical performance of the detection reagent. However, the range of antibody affinities of the peptides that could produce useful ELISA detection reagents is quite large since even with an affinity of ~0.25 μ M (K_D), P3-HRP-streptavidin was still a decent functional detection reagent.

The goal of the experiment illustrated in Fig. 5 was to demonstrate compatibility of peptide-based reagents with different means of the capture of the antibody on the surface of microplate well. The capture by species specific (anti-goat in this case) anti-IgG produced assays with excellent analytical performance using either biotin-P1-HRPstreptavidin (Fig. 5A) or digoxigenin-P1-HRP-anti-digoxigenin (Fig. 5B). The corresponding LODs were 50 and 54 pg/well, respectively, very similar to what was observed with protein G as a capture agent (Fig. 3B). The immobilization of the anti-Troponin I antibody by non-specific adsorption on a polystyrene microplate resulted in an assay that exhibited much worse analytical properties (Supplementary Fig. S4).

3.3. Peptide-based capture of the antibody for ELISA detection

Peptide ligands immobilized on solid surface were used for antibody capture to enable subsequent detection (for example, in peptide arrays [16]). We thus tested how analytical performance of peptide-based assays described above compared to an assay where the same peptides were used to capture the antibody in the wells of a microplate. We functionalized commercially available neutravidin-coated microplates with biotinylated P1, P2 and P3 peptides and performed ELISA assays where the antibodies were captured by the peptides immobilized in the microplate wells and were then detected by species-specific (anti-goat) anti-IgG HRPconjugated antibody. We first varied the amount of peptide immobilized in the wells to determine the minimal concentration necessary for maximal ELISA signals (Supplementary Fig. S5). Identical ELISA signals were observed in the range of 10 to 250 nM biotinylated peptide used in the immobilization reaction. We thus used 25 nM biotinylated peptides for immobilization in all subsequent experiments. Functionalizing neutravidin-covered plate with all three peptides (P1, P2 and P3) produced reproducible ELISA signals whereas for a plate covered with scrambled P1 peptide no signal over background was observed (Fig. 6A). The signals observed for the three peptides that differ in the affinity for the anti-Troponin I antibody decreased with the decrease of peptide affinity (Fig. 6A). However, compared to an analogous experiment with peptide-based detection reagents (Fig. 2A), the decrease in signal intensity was much smaller. Apparently, the efficiency of the capture of antibody by peptides immobilized on the surface of the plate is less sensitive to the binding affinity of the peptide ligand compared to when it is a functional part of a detection reagent. It is not clear why this difference in sensitivity of the assay performance to peptide binding affinity is observed. One possible explanation might be that a dense deposition of peptides on the solid surface could provide more opportunities for bivalent (more stable) binding of the antibody. Experiments testing specificity of the assay showed no significant cross reactivity at the same or 10x higher amount of the control antibodies except (as also was observed in case of peptide-based detection reagent (Fig. 2B)) a small increase of the signal observed at 10x of antibody specific to peptide 16–26 of Troponin I.

Analytical properties of the assay involving the capture of the antibody by immobilized P1 peptide (Fig. 7A) was very similar to what we observed for biotin-P1-HRP-streptavidin (Fig. 3A). The estimated LOD was 130 pg/well which was slightly worse compared to an assay with biotin- P1-HRP-streptavidin as a detection reagent (Fig. 3A). The assay worked well for detecting anti-Troponin I antibody spiked into the serum (Fig. 7B). Compared to detection in buffer (Fig. 7A), the background was higher and the reproducibility of the signal at a given antibody concentration was somewhat worse but the LOD was only ~2-fold higher (297 pg/well; Fig. 7B). This is a better analytical performance in serum compared to the results in Fig. 4A. Better performance of the assay involving the specific capture was to be expected as this design avoids the problem of saturation of the surface with IgG present in serum in high concentration. Similar LODs observed in buffer (Fig. 7A) and serum (Fig. 7B) indicated that surface saturation with IgG rather than competition between IgG in serum and target antibody for binding to peptide ligand was the main reason for much worse LOD in serum observed in experiments illustrated in Fig. 4.

4. Conclusions

Our data validate a simple experimental procedure that allows preparation of antibody specific detection reagents. These reagents are prepared using commonly available relatively inexpensive detection reagents. Peptide-based detection reagents can be used to prepare specific and sensitive ELISA assays for detecting antibodies. The reagents are prepared by simple mixing of appropriately tagged (for example, biotinylated) antibody-specific peptide ligand with a non-specific detection reagent (for example, HRP-streptavidin) or capture surface (for example, neutravidin-coated microplate). A simple mix-and-match approach could be thus used to prepare reagents specific to any antibody as long as a peptide ligand for such antibody is available. Peptide-based detection reagents exhibited very similar analytical performance compared to the assays where the peptides were used for antibody-specific capture in a microplate well. Peptides covering a wide range of antibody binding affinities produced functional ELISA reagents indicating that tight-binding peptide ligands corresponding to linear epitopes (as, for example, P1 peptide) as well as weaker affinity peptide mimotopes mimicking complex epitopes (or non-protein antigens) could be used to prepare ELISA reagents. Additional research (currently under way in our laboratory) will test this predicted wide practical utility of peptidebased ligands as ELISA detection reagents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: This work was supported by the National Institutes of Health [grant number 5R01GM109974].

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1. Specific ELISA antibody detection reagents can be produced by a simple mixing of appropriately functionalized short peptide ligands specific for target antibodies with common nonspecific detection reagents (for example, horseradish peroxidase-streptavidin conjugate).
2. One nonspecific detection reagent can be readily transformed into many different specific ELISA reagents by using different peptide ligands with specificities for desired target antibodies.
3. ELISA assays utilizing these detection reagents exhibit excellent analytical properties suggesting their practical utility for research or diagnostic applications.

Fig. 1.

(A) Peptide-based ELISA reagents for antibody detection. Antibody capture in microplate wells could involve nonspecific adsorption on polystyrene or capture in wells coated with protein G are target antibody species-specific anti-IgG. (B) Peptide-based capture of antibodies for ELISA detection.

Fig. 2.

(A) Comparison of ELISA signals for streptavidin-HRP as detection reagent paired with biotinylated P1, P2, P3 or scrambled P1 peptides. Protein G covered microplate was used for capture of anti-Troponin I antibody (7.5 ng). (B) Specificity of peptide-based ELISA detection reagent. ELISA signals using P1(biotin)-streptavidin-HRP for anti-Troponin I antibody (3 ng) are compared to signals obtained with the same and 10x higher amounts of indicated control antibodies. P1(biotin)-streptavidin-HRP was used as detection reagent on protein G microplate.

Fig. 3. ELISA signals as a function of anti-Troponin I antibody concentration. P1(biotin)-streptavidin-HRP (A) or P1(dig)-(anti-DIG)IgG-HRP (B) were used for detection on protein G microplate. Solid lines represent linear regression (slope=0.971+/-0.008, y-intercept=0.029+/-0.005, $r^2=0.99$ for panel A; slope=0.552+/-0.012, y-intercept=0.150+/-0.031, $r^2=0.99$ for panel B).

Fig. 4.

(A) ELISA signals as a function of anti-Troponin I antibody concentration in serum. Indicated concentrations of anti-Troponin I antibody were diluted in 25% human serum (serum diluted 1:4 with buffer). P1(biotin)-streptavidin-HRP were used for detection on protein G microplate. Solid line represents linear regression (slope= 0.019 ± 0.001 , y-intercept= 0.080 ± 0.020 , $r^2=0.99$) (B) Detection of anti-Troponin I antibody using P3(biotin)-streptavidin-HRP, a detection reagent utilizing peptide (P3) with ~50-fold lower antibody binding affinity compared to P1. Solid line represents a fit to a quadratic

polynomial ($y=y_0+a*x+b*x^2$; $y_0=0.039\pm0.010$, $a=0.047\pm0.003$, $b=0.0016\pm0.0002$, $r^2=0.99$).

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Fig. 5. ELISA signals as a function of anti-Troponin I antibody concentration. P1(biotin)-streptavidin-HRP (A) or P1(dig)-(anti-DIG)IgG-HRP (B) were used for detection on microplate functionalized with anti-goat antibody. Solid lines represent linear regression (slope=0.970+/0.015, y-intercept=0.020+/-0.010, $r^2=0.99$ for panel A; slope=0.938+/-0.008, y-intercept=0.062+/- 0.005, $r^2=0.99$ for panel B).

Fig. 6.

(A) Comparison of ELISA signals for biotinylated P1, P2, P3 or scrambled P1 peptides used for capture of anti-Troponin I antibody (7.5 ng) on neutravidin microplate. (B) Specificity of antibody capture on neutravidin plate functionalized with P1(biotin). ELISA signal for indicated amount of anti-Troponin I antibody is compared to signals obtained with the same and 10x higher concentrations of control antibodies. Control a: antibody against peptide 2 (PAPIRRSSNY) of human Troponin I; control b: antibody against phosphorylated human Troponin I; control c: antibody against human p53. P1(biotin)-streptavidin-HRP was used as

detection reagent on protein G microplate. Captured antibodies were detected with anti-goat-HRP.

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Fig. 7.

ELISA signals as a function of anti-Troponin I antibody concentration in buffer (A) and in 25 % serum (B). anti-Troponin I antibody was captured on neutravidin plate functionalized with P1(biotin) and was detected with anti-goat-HRP. Solid lines represent linear regression (slope=0.364 $\pm$ 0.008, y-intercept=0.042 $\pm$ 0.005, r^2 =0.99 for panel A; slope=0.238 $\pm$ 0.007, y-intercept=0.318 $\pm$ 0.005, r^2 =0.99 for panel B)