Corning® Medium and High Binding ELISA Microplates for Select Target Size Binding Assays

Application Note

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Introduction

Corning Medium Binding and High Binding surfaces are hydrophobic, non-treated polystyrene surfaces that bind biomolecules through passive adsorption. Medium Binding surfaces are ideal for large (>20kD), hydrophobic biomolecules and capture approximately 100 to 200 ng IgG/cm^2. On the other hand, High Binding surfaces are designed to facilitate binding of medium (>10kD) and large biomolecules with ionic groups and/or hydrophobic regions. The High Binding surface has capacity to bind up to 500 ng IgG/cm^2 which makes it ideal for enzyme-linked immunoassay (ELISA). The present study demonstrates the functionality of the Medium Binding and High Binding ELISA microplates by utilizing a range of target sizes up to 150 kDa: Angiotensin II (1.05 kDa), Insulin (5.8 kDa), Protein A (45 kDa), and IgG1 (150 kDa). Preferential surface chemistry for specific target size ranges was established by comparing the binding of each protein in Corning Medium Binding and High Binding ELISA 96-well microplates.

Materials/Methods

Both Medium Binding (Corning Cat. No. 9017) and High Binding (Corning Cat. No. 9018) 96-well microplates were employed to assess the size threshold (kDa) and quantity of bound protein. All target proteins were diluted in Dulbecco's Phosphate Buffered Saline (DPBS; Corning Cat. No. 21-031-CM) to the following concentrations: 5 µg/mL Human Angiotensin II (MilliporeSigma Cat. No. A9525-5MG), 0.4 µg/mL Recombinant Human Insulin (MilliporeSigma Cat. No. 91077C), 1.5 µg/mL Protein A (MilliporeSigma Cat. No. P7837), and 2.5 µg/mL Mouse IgG1 Isotype Control (Thermo Fisher Cat. No. 02-6100). Angiotensin II, Insulin, and Protein A were serially diluted 1:2 in DPBS in a dilution reservoir (Corning Cat. No. 4877) for an 8-point concentration response series. IgG1 was serially diluted 1:3 in DPBS for an 8-point concentration response series. For each replicate of the study, 2 plates of each Medium Binding and High Binding 96-well microplates were prepared with 150 µL DPBS control or protein dilution per well. Plates were then foil-sealed (Corning Cat. No. 6570) and incubated for 1 hour at room temperature.

After passive adsorption, the plates were washed with 200 µL/well of 1X wash buffer that was prepared by dilution of 10X TWEEN® 20 washing buffer (Fisher Scientific Cat. No. AAJ63314AP) in Type 2 water. The plates were then blocked with 150 µL/well of block buffer [1X wash buffer + 3% Bovine Serum Albumin (BSA; MilliporeSigma Cat. No. A9576-50ML)] for 1 hour at room temperature. During this time, primary antibodies were diluted in 1X wash buffer to the following concentrations: 10 µg/mL Angiotensin II Monoclonal Antibody (Thermo Fisher Cat. No. MA1-82996), 0.68 µg/mL Insulin Monoclonal Antibody (Thermo Fisher Cat. No. MA122711), and 1 µg/mL Mouse IgG1 Isotype Control (Thermo Fisher Cat. No. 02-6100) for Protein A detection. Blocking buffer was replaced with 100 µL/well of diluted primary antibody (or 1X wash buffer alone for Mouse IgG1), and incubated for an additional 1 hour at room temperature. The secondary antibody, Goat Anti-mouse IgG poly-horseradish peroxidase (HRP; Thermo Fisher Cat. No. 32230), was prepared by diluting stock 1:1,000 in 1X wash buffer. Each plate was washed 3X with 100 µL/well of wash buffer following primary incubation. Wash buffer was replaced with 100 µL/well of diluted secondary antibody, and the plate was again incubated for 1 hour at room temperature. Next, the washing steps above were repeated. TMB Working Substrate Solution (TMB Microwell Peroxidase Substrate Kit; VWR Cat. No. 95059-154) was prepared by adding equal amounts of reagent A and reagent B. The final wash solution was decanted, replaced with 100 µL/well of TMB Working Substrate Solution, and the plate was incubated for 5 minutes at room temperature in the dark on a plate rotator. The detection reaction was stopped with 100 µL/well of TMB Stop Solution (VWR Cat. No. 95059-200). Well solutions were mixed by brief (15-30 seconds) agitation on a shaking platform. Upon reaction with HRP conjugates, Substrate Solution develops a deep blue color with peak absorbance at 650 nm. Addition of Stop Solution acidifies the reaction, turning the solutions yellow with a peak absorbance at 450 nm (Abs_{450}). Abs_{450} was detected using a PerkinElmer Envision™ Multilabel Plate Reader.

Results/Discussion

The Corning Medium Binding microplates provide a non-treated polystyrene surface that is hydrophobic in nature, resulting in a natural affinity for biomolecules with large hydrophobic regions that can bind via passive interactions. This provides an ideal surface condition when isolating larger (>20kD) biomolecules such as immunoglobulins that have sizeable hydrophobic regions. Corning's High Binding surface binds medium (>10kD) and large

biomolecules that possess ionic groups and/or hydrophobic regions. Carboxyl groups present in the High Binding surface enable ionic interaction for more effective immobilization. This further allows the binding of detectable levels of smaller biomolecules.

In this study, the affinity of Corning® Medium and High Binding microplates for biomolecules was investigated with proteins ranging from 1.05 kDa (Angiotensin II) to 150 kDa (IgG1). Briefly, affinity was assayed by ELISA with detection using the TMB Microwell Peroxidase Substrate System with TMB Stop Solution. With this system, Abs_{450} is representative of the relative amount of bound protein and was used to quantify protein binding. The High Binding ELISA microplates displayed a higher average Abs_{450} value compared to the Medium Binding ELISA microplates across all four proteins analyzed (Figures 1, 2, 3, 4). With Angiotensin II, there was no observable difference in Abs_{450} from the baseline in the Medium Binding microplates at any concentration tested. By comparison, absorbance in High Binding microplates was close to 2 at the highest concentration of Angiotensin II (Figure 1). As evident in Figures 2 and 3, there was a minor increase (+0.2) in Abs_{450} for the highest concentrations of Insulin and Protein A in the Medium Binding microplates. The High Binding microplates exhibited a detectable difference from the baseline (+0.2), begin-

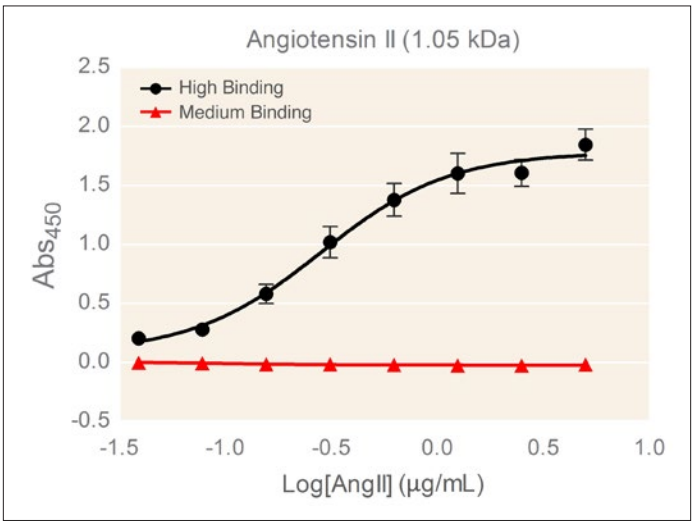

Figure 1. High Binding ELISA microplates exhibited higher binding capacity for Angiotensin II. A concentration series of Angiotensin II ranging from 39 ng/mL to 5 µg/mL was added to each ELISA microplate, and the amount of bound Angiotensin II was measured as Abs_{450} (Mean + SD).

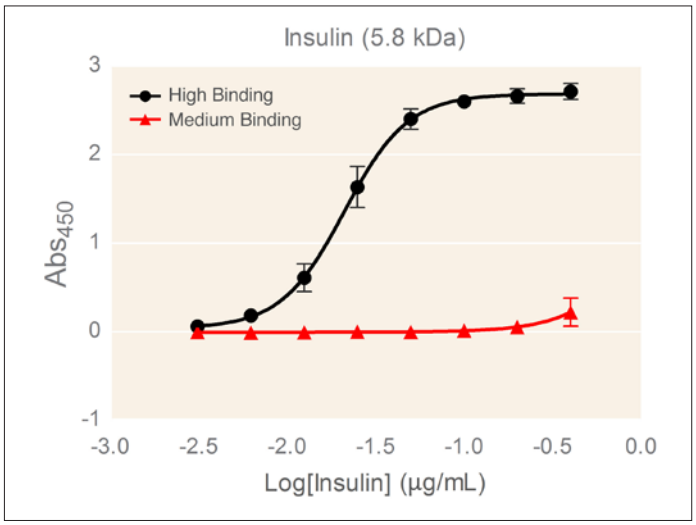

Figure 2. High Binding ELISA microplates exhibited higher binding capacity for Insulin. A concentration series of Insulin ranging from 3.125 ng/mL to 0.4 µg/mL was added to each ELISA microplate, and the amount of bound Insulin was measured as Abs_{450} (Mean + SD).

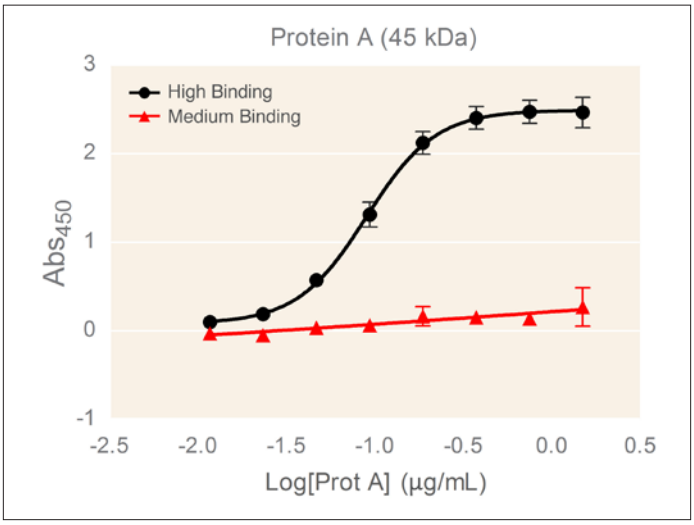

Figure 3. High Binding ELISA microplates exhibited higher binding capacity for Protein A. A concentration series of Protein A ranging from 11.7 ng/mL to 1.5 µg/mL was added to each ELISA microplate and the amount of bound Protein A was measured as Abs_{450} (Mean + SD).

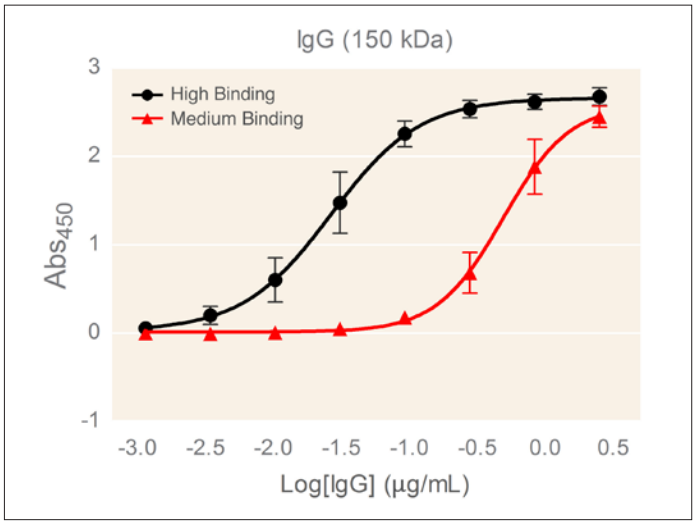

Figure 4. High Bind ELISA microplates exhibited higher binding capacity for IgG1. A concentration series of IgG1 ranging from 1.14 ng/mL to 2.5 µg/mL was added to each ELISA microplate and the amount of bound IgG1 was measured as Abs_{450} (Mean + SD).

ning at the lowest concentrations of Insulin and Protein A, to upwards of 2.5 at the highest concentrations of these proteins. Importantly, it was possible to capture binding of all the tested proteins on the High Binding microplates regardless of size. As shown in Figure 4, the Medium Binding microplate was only able to retain IgG1 protein, consistent with its optimal affinity for biomolecules of larger size (i.e., IgG). In addition, the IgG1 binding on the High Binding microplates was significantly shifted towards lower concentrations, with maximum binding occurring at 1.5 Log lower than the highest concentration on Medium Binding microplates. At the highest concentration of IgG1 tested, Medium Binding and High Binding microplates exhibited similar levels of IgG1 binding.

Conclusions

Corning® Medium Binding ELISA microplates provide an ideal surface for immobilization of larger proteins such as immunoglobulins that offer regions of hydrophobicity for passive adsorption binding. Corning High Binding ELISA microplates permit the immobilization of both smaller and larger biomolecules that can be bound through hydrophobic and/or ionic interactions, ranging from sizeable immunoglobulins to smaller molecules such as Angiotensin II peptide. The considerable range in size of biomolecules that can be captured by passive adsorption on the High Binding surface provides assay flexibility and an increased sensitivity for detecting lower concentrations of large proteins bound, relative to the Medium Binding surface.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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