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

Application Note

Audrey B. Bergeron, Christopher J. Bortz, and Ann Rossi, Ph.D.
Corning Incorporated, Life Sciences
Kennebunk, ME USA

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/cm². 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/cm² 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 in 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. 10X TWEEN® 20 washing buffer (Fisher Scientific Cat. No. 901844-25)).

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, $\text{Abs}_{450}$ 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 $\text{Abs}_{450}$ 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 $\text{Abs}_{450}$ 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 $\text{Abs}_{450}$ 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 $\text{Abs}_{450}$ (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 $\text{Abs}_{450}$ (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 $\text{Abs}_{450}$ (Mean + SD).

**Figure 4.** High Binding 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 $\text{Abs}_{450}$ (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.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.