Corning® Carbo-BIND Microplates for Enhanced Carbohydrate Binding Assays

Application Note

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Introduction

Corning Carbo-BIND surfaces contain hydrazide groups that covalently couple to carbohydrate (CHO) groups, making them ideal for assays requiring site-directed orientation of biomolecules such as oxidized antibodies, periodate-activated carbohydrates, or glycosylated proteins. With 1 x 10^{14}/cm^2 reactive sites, the Carbo-BIND surface demonstrates a specific binding preference for these biomolecules. The present study demonstrates this specific binding preference by utilizing alkaline phosphatase, which is commonly used to label biomolecules as its enzymatic activity is easily detected in colorimetric analysis. Specific binding was demonstrated by comparing the signal window between periodate-activated alkaline phosphatase and non-activated alkaline phosphatase on 96-well Carbo-BIND ELISA microplates relative to that obtained with Corning 96-well High Bind microplates, which have a slightly ionic surface for passive adsorption. In addition, the binding limits of both surfaces were determined from a concentration curve of activated alkaline phosphatase.

Materials/Methods

In order to compare the specific binding of periodate-activated alkaline phosphatase, Carbo-BIND 96-well microplates (Corning Cat. No. 2507) and High Bind 96-well microplates (Corning Cat. No. 9018) were used. Alkaline phosphatase (Thermo Fisher Cat. No. 31391) was diluted to 4.0 µg/mL in 10 mM sodium acetate buffer pH 5.5 that was prepared from 3 M sodium acetate (Sigma Cat. No. S7899-100ML). To activate alkaline phosphatase, sodium m-Periodate (Sigma Cat. No. S-1878-25G) was dissolved into 4.0 µg/mL alkaline phosphatase at a ratio of 3.2 mg/mL and incubated for 15 minutes in the dark at room temperature. After incubation, the activated alkaline phosphatase was used immediately for covalent coupling.

For covalent coupling, a high concentration of activated and non-activated alkaline phosphatase was prepared at 1.3 µg/mL. An 8-point dose series of activated alkaline phosphatase was also prepared with a top concentration of 4 µg/mL serially diluted 1 to 3 in 10 mM sodium acetate buffer pH 5.5. To each well of two Carbo-BIND and High Bind 96-well microplates, 100 µL were added of activated alkaline phosphatase, non-activated alkaline phosphatase, or buffer. The microplates were foil-sealed (Corning Cat. No. 6570) and incubated for 1 hour at room temperature. After incubation, the microplates were washed 4 times with 200 µL/well of Wash Buffer that was prepared by diluting 10X Wash Buffer [PBS with 1% TWEEN® 20] (Thermo Fisher Cat. No. AAJ63314AP) ten-fold in reagent grade type 2 water. The microplates were then blocked with 100 µL/well of Block Buffer [Wash Buffer supplemented with 2% Bovine Serum Albumin solution (BSA; Sigma Cat. No. A9576-50ML)] for 30 minutes at room temperature.

After incubation, Block Buffer was replaced with 100 µL/well of SeraCare BluePhos® Phosphatase for ELISA Working Substrate Solution (Thermo Fisher Cat. No. 50-674-54) and incubated for 10 minutes in the dark at room temperature. The reaction was stopped with 100 µL/well of BluePhos Stop Solution prepared from 10X stock (Thermo Fisher Cat. No. 50-674-57) and absorbance at 620 nm was detected using a PerkinElmer Envision™ Multilabel Plate Reader.

Results/Discussion

The Corning Carbo-BIND hydrazide surface enables covalent coupling to periodate-activated carbohydrates and glycosylated biomolecules, enhancing specific binding and detection of these types of biomolecules. On the other hand, the High Bind ELISA microplates, used here as the control for non-site-directed binding, have a hydrophobic surface modified with a small number of ionic carboxyl groups that results in a slightly ionic surface for passive adsorption. In this study, the specific binding of periodate-activated alkaline phosphatase to Carbo-BIND and High Bind ELISA microplates was monitored by the addition of a high concentration (1.3 µg/mL) of activated and non-activated alkaline phosphatase. The relative amount of alkaline phosphatase that bound to the microplates was quantified using the SeraCare BluePhos® Microwell Phosphatase Substrate System. The reaction of alkaline phosphatase with the substrate, which is a form of 5-bromo-4-chloro-3-indolyl phosphate (BCIP), yields a deep blue product with peak absorbance at 620 nm (Abs_{620}). After addition of BluePhos Stop solution to quench the reaction, Abs_{620} is representative of the relative amount of bound alkaline phosphatase.

Although the High Bind ELISA microplates displayed an average Abs_{620} value for activated alkaline phosphatase that was significantly higher than the average Abs_{620} value for non-activated alkaline phosphatase, the average values were only 1.3-fold apart: 0.74 for non-activated and 0.97 for activated (Figure 1). This suggests that much of the binding for activated alkaline phosphatase observed with the High Bind microplate is not specific to the activated site. In contrast, the Carbo-BIND ELISA microplates displayed Abs_{620} values for activated alkaline phosphatase (average of 2.32) that were nearly 40-fold higher

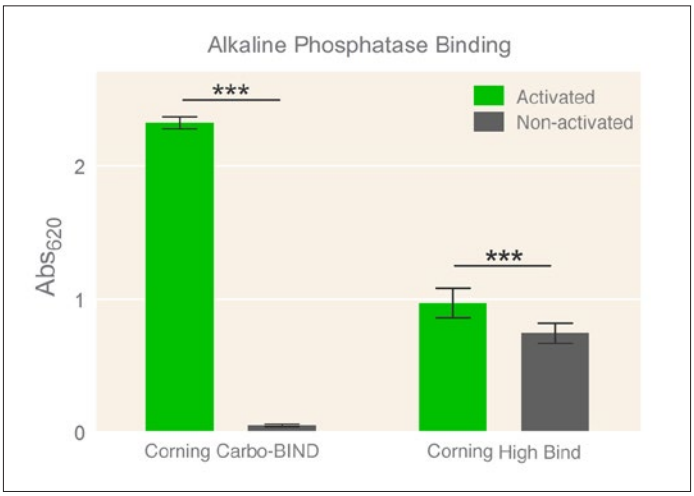

Figure 1. Corning Carbo-BIND ELISA microplates show specific binding of activated alkaline phosphatase. Corning High Bind ELISA microplates (right) displayed an average Abs_{620} of 0.97 ± 0.11 for activated alkaline phosphatase (green) and 0.74 ± 08 for non-activated alkaline phosphatase (gray), which were significantly different. However, Carbo-BIND ELISA microplates (left) displayed a higher average Abs_{620} of 2.32 ± 0.04 for activated alkaline phosphatase and a lower average Abs_{620} of 0.06 ± 0.01 for non-activated alkaline phosphatase. Error bars represent standard deviation (SD). ***p<0.001 in two-way ANOVA with Bonferroni post hoc test; n = 6.

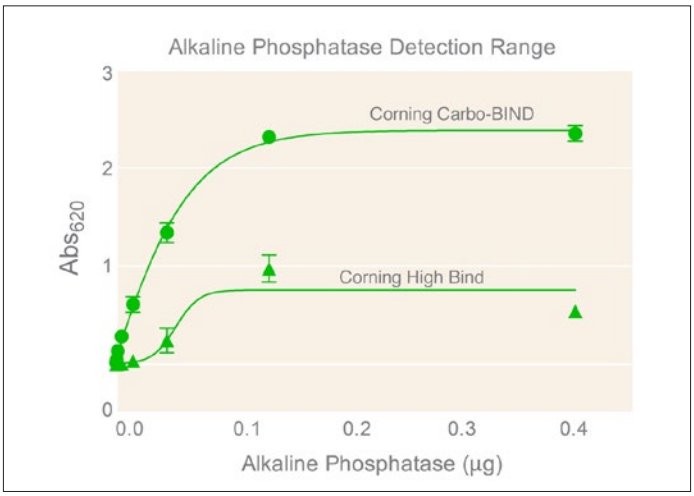

Figure 2. Corning Carbo-BIND ELISA microplates exhibited higher binding capabilities of activated alkaline phosphatase. A concentration series of activated alkaline phosphatase ranging from 0.18 to 400 ng per well was added to each ELISA microplate and the amount of alkaline phosphatase that bound was detected using a BluePhos® substrate reaction. The Carbo-BIND surface bound and retained more activated alkaline phosphatase than the Corning High Bind surface, indicated by larger Abs_{620} values. Error bars represent SD.

than the Abs_{620} values for non-activated alkaline phosphatase (average of 0.06) (Figure 1) which demonstrates the site specific binding of periodate-activated alkaline phosphatase to the Corning® Carbo-BIND ELISA microplate.

A concentration series of activated alkaline phosphatase was prepared in order to understand the limits of the surfaces for binding detectable amounts of activated alkaline phosphatase (Figure 2). The Carbo-BIND ELISA microplate displayed Abs_{620} values that plateaued at ~2.3 with the addition of 130 ng of activated alkaline phosphatase per well. The High Bind ELISA microplate also displayed Abs_{620} values that plateaued at 130 ng of activated alkaline phosphatase per well. However, the peak Abs_{620} values of the High Bind microplate was only 0.97. This suggests that the Carbo-BIND ELISA microplates successfully captured more of the alkaline phosphatase that was added to the wells.

Conclusions

Corning Carbo-BIND ELISA microplates provide an ideal surface for site-directed binding of carbohydrate groups such as those found on oxidized antibodies, periodate-activated carbohydrates, or glycosylated proteins. Although these biomolecules can be immobilized via passive adsorption to polystyrene as exhibited here with the Corning High Bind microplate using periodate-activated and non-activated alkaline phosphatase, the Carbo-BIND microplate exhibits preferential binding of periodate-activated alkaline phosphatase that is captured at detectable levels from lower concentrations.

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