Tech Note
No. 59

C8 Black Thermo Scientific Nunc LockWell FluoroNunc MaxiSorp and PolySorp for Fluorescence Detection

The new black Thermo Scientific Nunc LockWell format with MaxiSorp and PolySorp wells have a recommended maximum volume of 350 µL. These breakable strips have letters and notches on each well for easy identification of individual wells. The strips are suitable for all commonly used automated equipment. The dense pigmentation of black Nunc™ LockWell™ modules minimizes background fluorescence. MaxiSorp™ is optimized for binding IgG (antibodies) and PolySorp™ for binding more hydrophobic molecules.

In this study we compare the performance of the black LockWell modules with similar products from other large suppliers: Competitors A, B and B low volume (total volume of 205 µL/well). Plate uniformity and binding capability are determined by monitoring the reaction of immobilized horseradish peroxidase (HRP) from coating with a mixture of rabbit IgG and HRP conjugated rabbit anti-mouse IgG. Three of each plate type were tested on three different days.

When comparing high binding surfaces, the highest binding capability and lowest relative standard deviation was found to be LockWell MaxiSorp and Competitor A (using coating volumes of 150 µL/well) (Fig. 1). Comparing the LockWell format to Competitor B low volume (using a coating volume of 100 µL/well) a very low relative standard deviation and high binding capability was found for the LockWell format. The binding capability of the LockWell MaxiSorp surface can easily be increased using a higher coating volume.

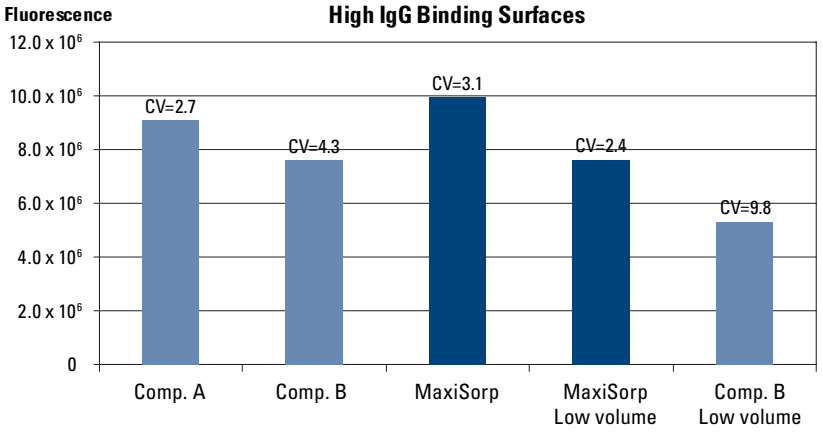

Fig. 1.
Fluorescence intensity (antibody binding capability) measured after performing antibody binding assay on different medium binding surfaces. Coating volume of Competitor A, B and MaxiSorp modules was 150 µL/well, and coating volume of PolySorp and competitor B low volume was 100 µL/well. Mean CV for tested module plates is shown above the respective binding capability column.

Assay

- Coating overnight at room temperature with antibody mixture (100 µL/well using low volume or 150 µL/well using standard 96 format).
- Wash three times with washing buffer.
- Addition of HPPA substrate (100 µL/well using low volume or 150 µL/well using standard 96 format).
- Addition of 50 µL/well sodium hydroxide after 14 minutes. The fluorescence was measured on EnVision 2101 using optimized fluorescence protocols with filter sets 340/405 and 485/535 nm.

Reagents

Antibody mixture consisting of 65 ng/mL HRP conjugated rabbit anti-mouse IgG and 10 µg/mL rabbit IgG, diluted in 0.05 M sodium carbonate buffer, pH 9.6.

Washing buffer: 0.15 M PBS, pH 7.2, with 0.05% detergent (Triton X for high binding surfaces and Tween 20 for medium binding surfaces).

Freshly prepared HPPA substrate: 2.5 mm 3-(p-hydroxyphenyl) propionic acid dissolved in 0.1 M TRIS buffer, pH 8.5, and 1.5 µL 30% hydrogen peroxide is added to 100 mL substrate.

Binding capability and relative standard deviations between medium binding surfaces was found to be comparable, using coating volumes of 150 µL/well (Fig. 2). Comparing the LockWell

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format to Competitor B low volume, a very low relative standard deviation is achieved using a coating volume at 100 µL/well. The binding capability when using the LockWell for low volume coatings is comparable to the Competitor B low volume. The binding capability of the LockWell PolySorp surface can easily be increased using a higher coating volume.

Fluorescence background measurements were performed using exitation and emmission filter sets 485/535 and 340/405 nm. The data are not shown, as it was found relatively low for all products, regardless of the surface type and format.

Conclusion

Data show high binding capability and high uniformity using fluorescence detection on black C8 LockWell FluoroNunc™, demonstrated for different coating volumes by IgG binding assay on both the MaxiSorp and PolySorp surfaces.

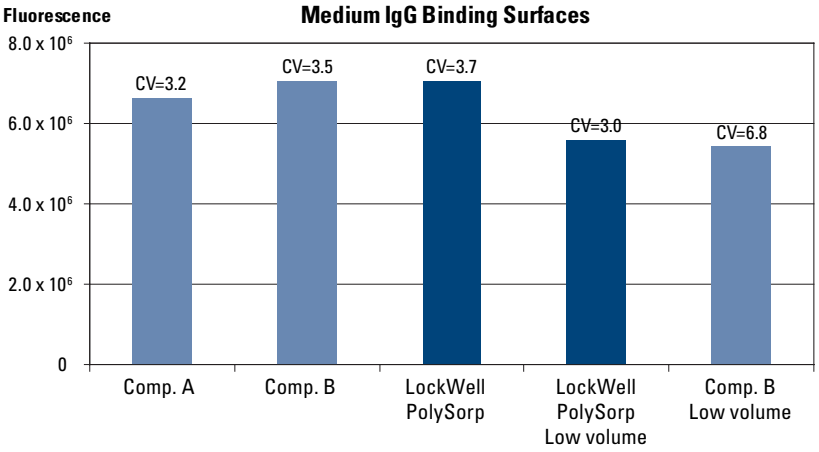

Fig. 2.
Fluorescence intensity (antibody binding capability) measured after performing antibody binding assay on different high medium surfaces. Coating volume of Competitor A, B and PolySorp modules was 150 µL/well, and coating volume of PolySorp and competitor B low volume was 100 µL/well. Mean CV for tested module plates is shown above each relevant binding capability column.

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