Thermo Scientific Nunc C8 White LockWell LumiNunc MaxiSorp and PolySorp for Luminescence Detection

Application Note

Key Words

Thermo Scientific™ Nunc™ LockWell™, Thermo Scientific™ Nunc™ MaxiSorp™ Surface, Thermo Scientific™ Nunc™ PolySorp Surface, Thermo Scientific™ Nunc™ LumiNunc™, luminescence detection, white module, competitive analysis.

Goal

The goal of this application note is to describe the benchmarking study of the Lockwell C8 white modules compared to similar products from other suppliers. The plates are tested for binding capacity and standard deviation. The application note conclude the Lockwell C8 white module to out-perform the compared plates when binding antibodies at different coating volumes in respect to binding capacity and lowest relative standard deviation.

The new white Nunc LockWell is available with Nunc MaxiSorp and Nunc PolySorp surfaces. The format is constructed as a breakable module with letters and notches on each well for easy identification and a maximum volume of 350 µL/well. The format makes the modules suitable for all commonly used automated equipment. The white Nunc LockWell modules are densely pigmented to obtain high reflection and minimize crosstalk. Nunc MaxiSorp is optimized for binding of IgG (antibodies), and Nunc PolySorp for binding more hydrophobic molecules.

The purpose of this study is to compare the performance of the white Nunc LockWell modules with similar products from other large suppliers: Competitor A, B and B low volume (total volume of 205 µL/well). Plate uniformity and binding capability are measured by detection of immobilized horseradish peroxidase (HRP) from coating with a mixture of rabbit IgG and HRP conjugated rabbit anti-mouse IgG.

Three plates of each type were tested on three independent days.

The highest binding capability and lowest relative standard deviation between high binding surfaces was found to be Nunc LockWell MaxiSorp using coating volumes of 150 µL/well (Fig. 1). Comparing the Nunc LockWell format to Competitor B low volume, using a coating volume of 100 µL/well, a very low relative standard deviation is still achieved with Nunc LockWell.

Also, when using the Nunc LockWell for low volume coatings, the binding capability is comparable to the Competitor B low volume. The binding capability of the Nunc LockWell MaxiSorp surface can easily be increased by using a higher coating volume.

Assay

Coating overnight at room temperature with antibody mixture (100 µL/well using low volume or 150 µL/well using standard 96 format).

Wash 3X with washing buffer.

Addition of luminol substrate (100 µL/well using low volume or 150 µL/well using standard 96 format).

Immediately after adding of substrate, the luminescence intensity is measured on EnVision 2101 using optimized ultra-sensitive luminescence protocols, reading time 0.1 sec.

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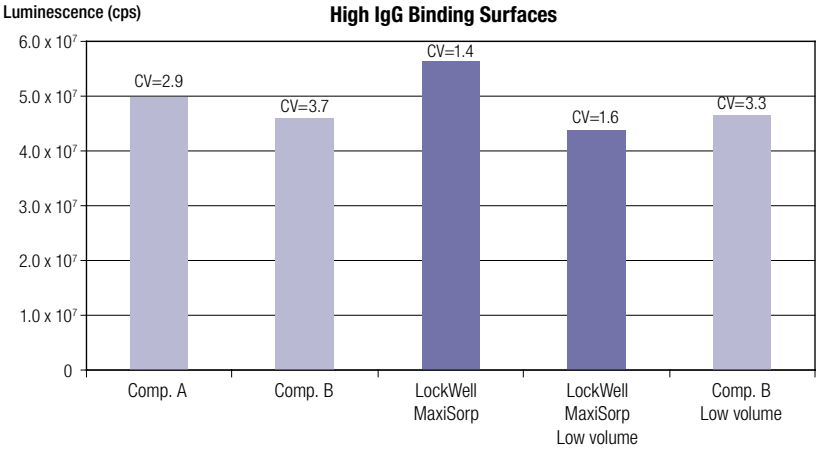

Fig. 1.

Luminescence signal (antibody binding capability) measured after performing antibody binding assay on different high binding surfaces. Coating volume of Competitor A, B and Nunc MaxiSorp modules was 150 µL/well, and coating volume of Nunc MaxiSorp and competitor B low volume was 100 µL/well. Mean CV for tested module plates is indicated above the respective binding capability column.

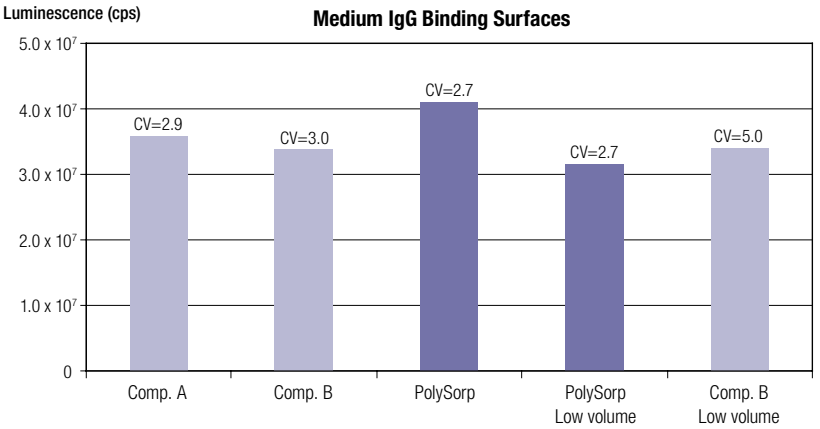

Fig. 2.

Luminescence signal (antibody binding capability) measured after performing antibody binding assay on different medium binding surfaces. Coating volume of Competitor A, B and Nunc 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 respective binding capability column.

Reagents

Antibody mixture consisting of 65 ng/mL HRP conjugated rabbit anti-mouse IgG P0260 and 10 µg/mL Rabbit IgG X0903, 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).

Luminol stock solution: 0.32 M 3-(p-aminophthalhydrazide) and 0.36 M 4-iodophenol dissolved in dimethyl sulfoxide.

Dilute both luminol stock solution and a 0.3 % hydrogen peroxide solution 1:250 in 0.1 M TRIS buffer, pH 8.5.

The highest binding capability between medium binding surfaces was found to be Nunc LockWell PolySorp using coating volumes of 150 µL/well (Fig. 2), while the relative standard deviations are comparable. Comparing the Nunc LockWell format to Competitor B low volume, a very low relative standard deviation is achieved using a coating volume of 100 µL/well. The binding capability when using the Nunc LockWell for low volume coatings is comparable to the Competitor B low volume. The binding capability of the Nunc LockWell PolySorp surface can easily be increased by using a higher coating volume.

Conclusion

Data show high binding capability and high uniformity using luminescence detection on white Thermo Scientific Nunc C8 LockWell LumiNunc, demonstrated for different coating volumes by IgG binding assay on both the Nunc MaxiSorp and Nunc PolySorp surfaces.

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