Tech Note
No. 58

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

The new white Thermo Scientific Nunc LockWell is available with Thermo Scientific Nunc MaxiSorp and 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 LockWell™ modules are densely pigmented to obtain high reflection and minimize crosstalk. MaxiSorp is optimized for binding of IgG (antibodies), and PolySorp for binding more hydrophobic molecules.

The purpose of this study is to compare the performance of the white 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 LockWell MaxiSorp 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 is still achieved with LockWell. Also, when using the LockWell for low volume coatings, the binding capability is comparable to the Competitor B low volume. The binding capability of the LockWell MaxiSorp surface can easily be increased by using a higher coating volume.

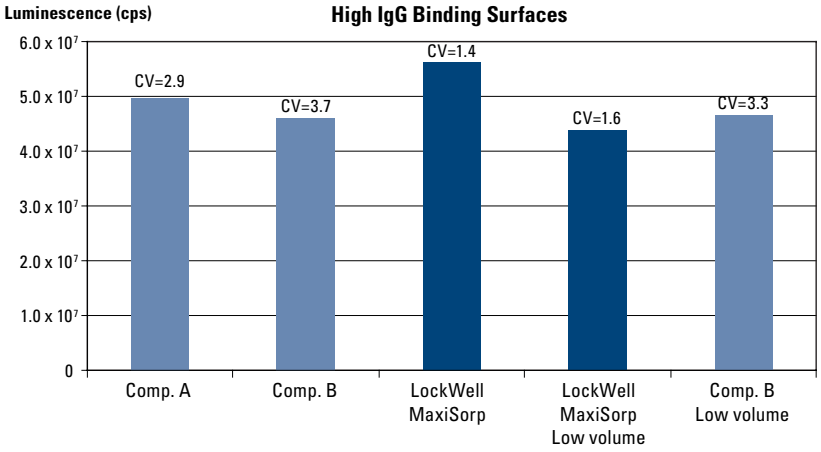

Fig. 1.
Luminescence signal (antibody binding capability) measured after performing antibody binding assay on different high binding surfaces. Coating volume of Competitor A, B and MaxiSorp modules was 150 µL/well, and coating volume of MaxiSorp and competitor B low volume was 100 µL/well. Mean CV for tested module plates is indicated 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 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.

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 LockWell PolySorp using coating volumes of 150 µL/well (Fig. 2), while

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the relative standard deviations are comparable. Comparing the 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 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 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 MaxiSorp and PolySorp surfaces.

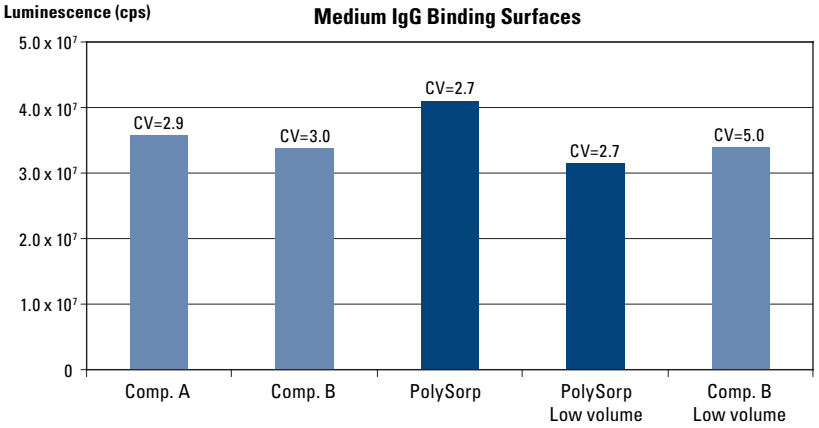

Fig. 2.
Luminescence signal (antibody binding capability) measured after performing antibody binding assay on different medium binding surfaces. Coating volume of Competitor A, B and PolySorp modules was 150 µL/well, and coating volume of PolySorp and competitor B low volume was 100 µL/well. Mean CV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

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