Performance of Thermo Scientific Nunc Immuno C8 StarWell

Application Note

The Nunc Immuno Module C8 StarWell, MaxiSorp or PolySorp surfaces, consists of Nunc MicroWells containing eight inner fins. As a result, the surface area is increased by a factor of approximately 1.5 when compared to standard, flat-bottom F-wells using reactant volumes ranging from 50 to 200 µL.

In a solid phase assay, e.g. ELISA, a larger surface/volume ratio implies a faster adsorption of molecules from the liquid phase. Thus, the primary advantage of a larger surface/volume ratio is that assay time can be reduced.

Nunc StarWell incubation times may be reduced by a factor of approximately 2.25. According to adsorption kinetics modelling [1], incubation times can be reduced by a factor equal to the square of the surface/volume increase factor, without reducing adsorption.

This Tech Note demonstrates Nunc StarWell vs. standard F-well performances for first layer, passive IgG adsorption according to the model [1]. The performance relationship holds for immobilization of any reactant in a solid phase bioassay sequence [1].

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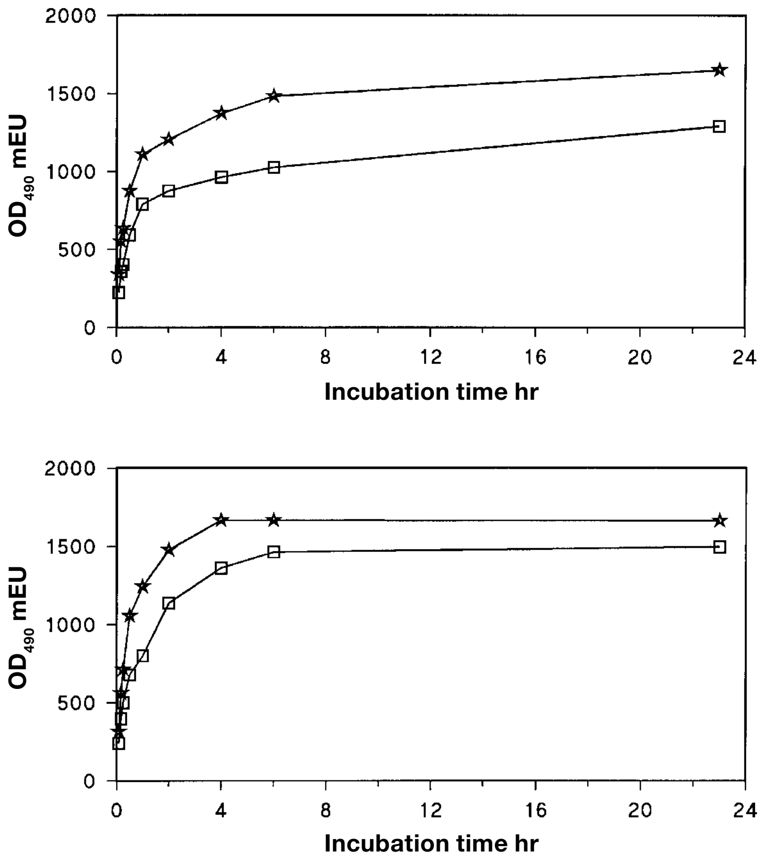


Fig. 1.

Data of IgG adsorption kinetics with Nunc MaxiSorp StarWell (★) and standard well (☐) using IgG:HRP conjugate (Dako P 128) and IgG (Dako Z 181) diluted in carbonate buffer, pH 9.6.

In one case, 150 µL/well of IgG:HRP conjugate was used in a dilution corresponding to 1% surface saturation of a standard well, i.e. $F_{\square} = 0.01$ (left). In another case, 150 µL/well of IgG + IgG:HRP (= 99+1) was used in a dilution corresponding to 100% standard well saturation, i.e. $F_{\square} = 1$ (right). F generally denotes the ratio between the number of supplied molecules and the number of molecules that can be adsorbed. The MaxiSorp IgG adsorption capacity has been estimated to 650 ng/cm^2 [2], and at 150 µL liquid volumes the Nunc StarWell and standard well surface/volume ratios are 1.5 x 8.3 and 8.3 cm^{-1} respectively [3].

After adsorption, the wells were washed 3 times with PBS + 0.2 M extra NaCl + 0.05% Triton X-100, pH 7.2. This was followed by substrate reaction with 150 µL H_2O_2 /OPD in phosphate-citrate buffer, pH 5.0, and addition of 110 µL 2N H_2SO_4 stopping solution, implying 260 µL reading volumes.

Fig. 2.

Relationship between OD increase and liquid volume with Nunc StarWell due to the fins' elevation of liquid heights.

This relationship is empirically found to be approximately linear in a semilogarithmic plot. The dashed regression line represents the relationship with Nunc MaxiSorp, and the dotted line the relationship with Nunc PolySorp. The solid line and the equation represent the average data (O). In the hydrophobic Nunc PolySorp wells, the liquid stands higher than in the hydrophilic MaxiSorp wells due to the downward liquid surface curvature in Nunc MaxiSorp. This gives rise to a higher Nunc MaxiSorp elevation percentage. Actual percentages are determined by multiplying or dividing the average calculation by 1.15 for Nunc MaxiSorp or Nunc PolySorp respectively. In the present case, using Nunc MaxiSorp and reading volumes of 260 µL, the elevation is approximately 7%. The Nunc StarWell readings must therefore be reduced by 7% to match the standard well readings for a comparative analysis of the respective adsorption performances (Figs. 3-5).

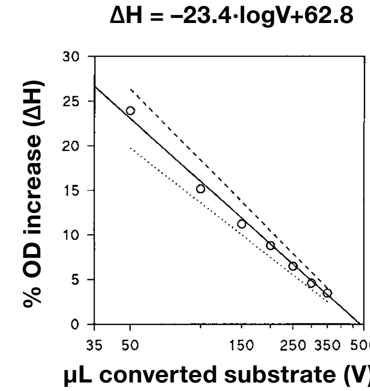


Fig. 3.

Simulations of the Fig. 1 data, adjusted according to Fig. 2, by the adsorption kinetics model [1], showing correlation between real and theoretical kinetics.

At molecular supplies corresponding to $F_{\square}$ = 1 (Fig. 3A), the Nunc StarWell adsorption acceleration is much larger than at molecular supplies corresponding to $F_{\square}$ = 0.01 (Fig. 3B). The reason for this is explained in Fig. 4. As an example, the levels of 75% adsorption of supplied molecules (red horizontal lines) are used for comparison of the Nunc StarWell vs. standard well performances.

Between the lines of equal adsorption percentage (75%) and equal incubation time (red vertical lines), the StarWell curves exhibit regimes, elaborated in Fig. 5, where both time reduction and adsorption increase can be obtained.

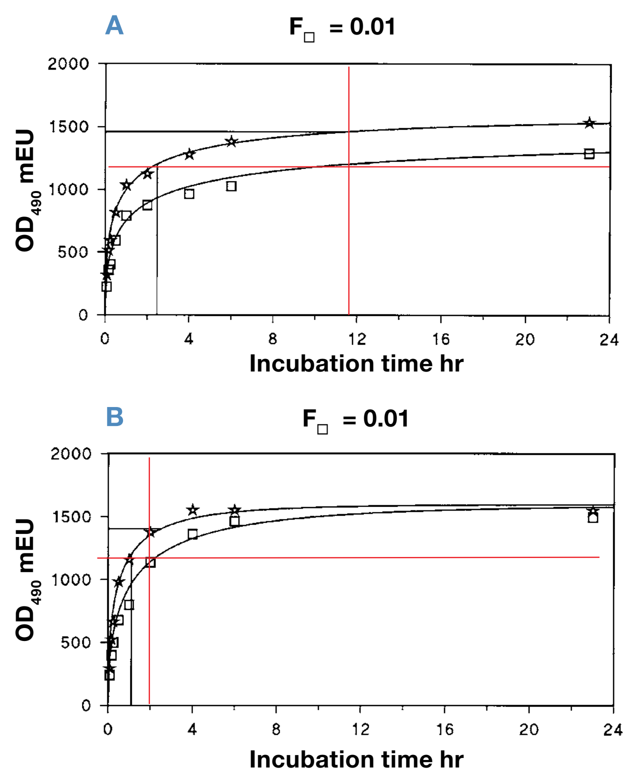


Fig. 4.

Model derived incubation times, T_{75}, for 75% adsorption of supplied molecules, as functions of F, in percent of the standard well T_{75} value at F = 1, with Nunc StarWell (lower curve) and standard well (upper curve).

The curves are almost constant for F values up to 0.1, because in this F regime, plenty of unoccupied sites are available on the surface. At F = 1, the curves assume maximum values, because the surfaces approximate saturation during adsorption; therefore, it will take a longer time for the last molecules to find the last unoccupied sites. In the present cases, where the molecular supplies correspond to $F_{\square}$ = 0.01 and 1, the corresponding Nunc StarWell values are 1.5 times smaller due to the 1.5 times larger surface area, i.e. $F_{\star}$ = 0.0067 and 0.67, respectively. Therefore, a relatively larger Nunc StarWell adsorption acceleration is obtained at the high F values (dashed lines) than at the low F values (dotted lines) – in accordance with the data in Fig. 3. Thus, the claimed incubation time reduction factor of 2.25 is valid for $F_{\square}$ values up to approximately 0.1. For $F_{\square}$ values ranging from 0.1 to 1, the factor will be larger.

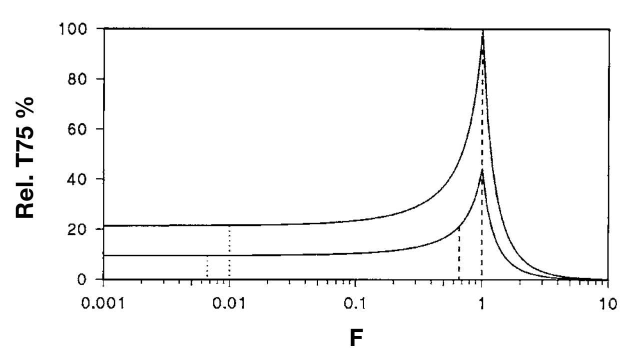
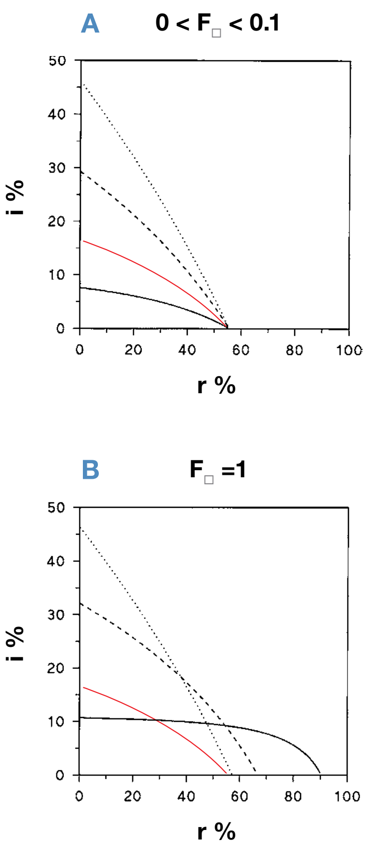


Fig. 5.

Model derived adsorption increase (i) vs. incubation time reduction (r) with Nunc StarWell compared to standard well for 10% (···), 50% (---), 75% (—) and 90% (—) adsorption of supplied molecules.

For $F_{\square}$ values up to 0.1 (Fig. 5A) the maximum time reduction is approximately constant for any adsorption level. At $F_{\square} = 1$ (Fig. 5B) the maximum time reduction increases with increasing adsorption level (Fig. 3). For $F_{\square}$ values ranging from 0.1 to 1, a gradual transition from the left to the right diagram curves will occur.

Between the extremes of maximum r (i = 0) and maximum i (r = 0), intermediate r and i values can be obtained with StarWell.



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