Application Note

Edge Effect in Thermo Scientific Nunc MicroWell ELISA

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Sometimes with ELISA performed in a Thermo Scientific Nunc MicroWell plate unexpectedly higher (or lower) optical densities (O.D.) are measured in the peripheral wells than in the central wells. This phenomenon is called ”edge effect”.

The most probable causes of this effect are illumination or temperature differences between the peripheral and the central wells.

Light may cause edge effect if the substrate is photosentitive (i.e. converted by light exposure) like the H^2O^2/OPD substrate in the peroxidase system. Thus, if strong light is coming from one side (e.g. sunlight from a window) during the substrate reaction, the peripheral wells closest to the light source may give elevated O.D. values.

Temperature difference, however, is the most common cause of edge effect.

Incubation at 37°C instead of room temperature is often used for shortening incubation times due to the fact that at higher temperatures the dissolved molecules move faster and will therefore reach the well surface sooner than at lower temperatures.

However, a common mistake is to use reactant liquids straight from a refrigerator and then incubate in a 37°C incubator (or at room temperature). Temperature changes of these magnitudes may, especially with short incubation times, destroy the assay homogeneity in Nunc™ MicroWell™ plates. The peripheral wells will normally be heated up first because of their position closest to the lower edge of the plate, which is in direct contact with the warm incubator shelf. Therefore, more reactant molecules may be immobilized in the peripheral wells, which may result in higher O.D. values in these wells, other things being equal.

The edge effect may be more pronounced if plates are stacked during incubation, especially in plates in the middle of the stack because their central wells are shielded from the warmer surroundings by the plates above and beneath.

To demonstrate a pronounced edge effect caused by temperature differences, a stack of 5 Thermo Scientific Nunc MaxiSorp plates with 4°C IgG:peroxidase conjugate, 200 µL per well, were incubated at 37°C for 30 minutes prior to substrate reaction. All the plates showed edge effect compared with a control plate with room temperature conjugate incubated at room temperature. The most pronounced effect was observed in the second bottom plate, the results of which are given in Fig. 1.

Even if temperature changes are avoided, a small temperature dependent edge effect may remain, which can be disturbing in critical assays when incubation times

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are short. Due to heat consumption by evaporation (which is assumed to be equal from all the wells in uncovered plates), the wells will be cooled down. However, the heat loss will be restored faster in peripheral wells than in central wells, thus producing temperature differences and possibly edge effect.

To avoid the above-mentioned problems, the following precautions should be taken:

1. Incubations should take place in subdued light or in the dark.
2. Reactant liquids (and plates) should be adjusted to the temperature intended for incubation.
3. Plates should be sealed with adhesive tape or placed in a 100% relative humidity environment during incubation.

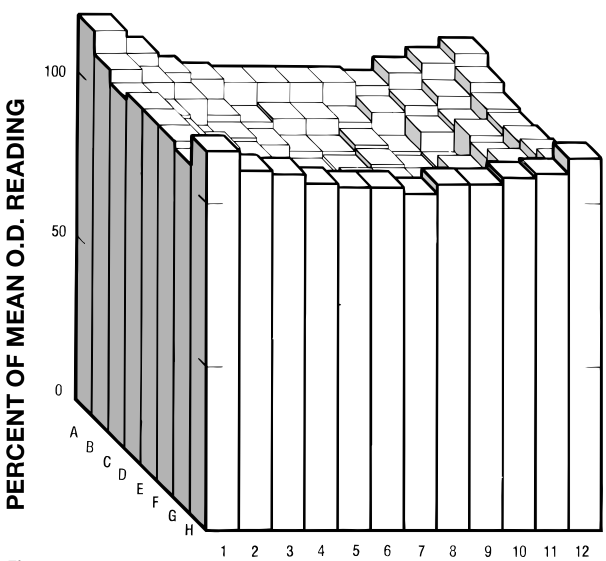


Fig. 1

Block diagram of the plate O.D. readings from H_2O_2/OPD substrate reactions in a MicroWell plate illustrating the edge effect after incubation with 4°C IgG:peroxidase conjugate at 37°C for 30 minutes. Each column represents the O.D. reading of the respective well in percent of the plate mean value (952 mEU). Note that the edge effect is most pronounced in the corner wells, A1 giving the maximum value = 118%, while the central well D6 gives the minimum value = 89%. See text for further explanation.

Adsorption Geometry for Solid Phase Assays

For optimal set-up of a solid phase assay it is essential to know the dimensional relationship between the "solid phase products" and the volumes of liquid.

Table 1 lists the geometric figures that correspond to certain volumes of liquid in Thermo Scientific Nunc solid phase products.

The size of the plastic area covered with liquid reflects the total binding capacity with that particular combination of vessel and volume of liquid and thus the total amount of e.g. IgG needed for a saturated coating of the surface covered.

The liquid height figures can be used to estimate the available free volume for possible addition of extra liquid (e.g. sulphuric acid for stopping the color reaction in ELISA) and to estimate the optimal thickness of the developed color layer (in MicroWell assays) when measured in a photometric MicroWell reader. In tube assays the final liquid height must be above the level of the transverse measuring light beam in the applied tube reader.

The area/volume ratio reflects the amount of reactant molecules that can be bound per mL of liquid used.

The higher the ratio is, the more molecules can be bound per mL liquid.

Using the plausible estimate that the surface can adsorb 400 ng IgG per cm², the approximate concentration S needed for saturation can be calculated from the area/volume ratio R: S=0.4x R µg IgG per mL.

For coating the TSP, the adsorption by the vessel into which the TSP is dipped should also be taken into consideration.

The ratio is also a relative measure of the mean distance that the dissolved reactant molecules have to travel to reach the solid phase and thus of the time needed for the molecules to be bound. In general, the higher the ratio is, the shorter the distance and the incubation time needed will be. The benefits of a high area/volume ratio prompted us to develop the Thermo Scientific Nunc Immuno StarTube with 6 fins in the bottom of the tube, thus increasing the area/volume ratio considerably compared to an ordinary tube. The relative increase is dependent on the volume of liquid added, i.e. the smaller the volume, the larger the increase.

Fig. 2 illustrates the favorable effects of the increased area/volume ratio when using the StarTube.

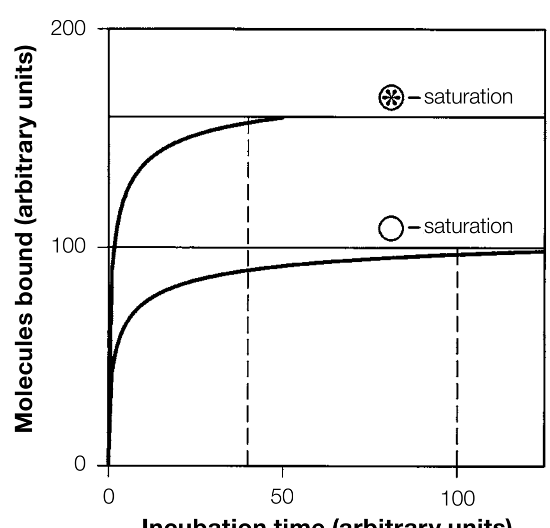


Fig. 2

Average adsorption curves showing the increase in number of bound molecules and the decrease in incubation time obtainable with 350 µL reactant volume by use of the 75x12 mm StarTube (⊛), compared to the ordinary 75x12 mm standard tube (◯)

The mutual relationship between these curves holds for the binding of every successive layer in the immuno assay sandwich.

Table 1

Corresponding figures relevant for designing solid phase assays in Thermo Scientific Nunc products.

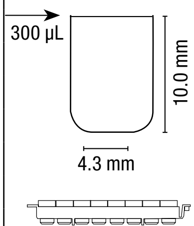
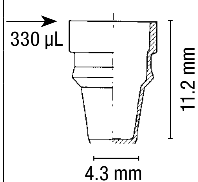
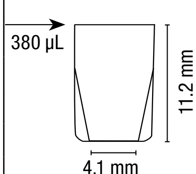
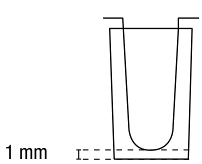
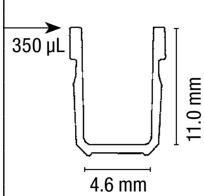
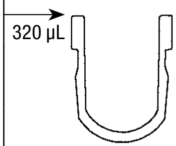
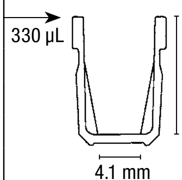
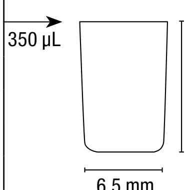
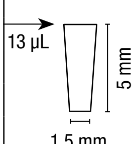
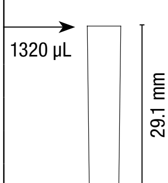
Product	Liquid volume, µL	Covered area, mm²	Liquid height, mm	Area/volume ratio, cm²/cm³
Nunc BreakApart Modules, C8				
	250	190	8.5	7.6
	200	159	6.9	7.9
	175	143	6.1	8.2
	150	127	5.2	8.5
	125	110	4.4	8.8
	100	94	3.5	9.4
	75	78	2.7	10.4
	50	61	1.8	12.2
Nunc NucleoLink Modules				
	330	234	11.2	7.1
	200	159	8.1	7.9
	100	96	5.2	9.6
	50	57	2.4	11.4
	25	37	1.6	14.8
Nunc StarWell Modules, C8				
	250	23	7.7	9.0
	200	198	6.3	9.9
	175	193	5.7	11.0
	150	166	4.9	11.0
	125	146	4.2	11.7
	100	125	3.4	12.5
	75	100	2.6	13.3
	50	72	1,8	14.4
Nunc TSP in MicroWell F				
	250	95	9.7	3.8
	200	75	7.6	3.8
	150	53	5.6	3.5
	125	41	4.6	3.3
	100	29	3.5	2.9
	75	20	2.8	2.4
	50	9	1.8	1.8
	50	55	3.4	11.0
Nunc LockWell Modules, C8				
	250	188	7.9	7.5
	200	157	6.4	7.8
	175	141	5.6	8.1
	150	126	4.8	8.4
	125	110	4.0	8.8
	100	94	3.2	9.4
	75	78	2.4	10.4
	50	62	1.6	12.5

Table 1 (cont'd.)

Product	Liquid volume, µL	Covered area, mm²	Liquid height, mm	Area/volume ratio, cm²/cm³
Nunc LockWell Modules, U8				
	250	178	8.6	7.1
	200	147	7.1	5.9
	175	132	6.3	7.5
	150	116	5.6	7.7
	125	101	4.8	8.0
	100	85	4.0	8.5
	75	69	3.2	9.2
	50	53	2.5	10.7
Nunc LockWell StarWell Modules, C8				
	250	244	8.4	9.8
	200	213	6.9	10.7
	175	196	6.2	11.2
	150	177	5.4	11.8
	125	156	4.5	12.5
	100	132	3.7	13.2
	75	105	2.8	14.0
	50	75	1.9	15.1
Nunc MicroWell Modules, C8, C12				
	250	185	8.3	7.4
	200	154	6.7	7.7
	150	122	5.1	8.1
	125	106	4.3	8.5
	100	90	3.5	9.0
	75	73	2.7	9.7
	50	56	1.8	11.2
Nunc 1536 Well Plates				
	12	32	4.6	26.7
	10	27	3.9	27.3
	8	23	3.2	28.0
	6	18	2.4	29.3
	4	113	1.6	31.5
	2	8	0.9	37.3
	1	5	0.5	47.2
Nunc 96 DeepWell Plates 1.0 mL				
	1200	649	26.9	5.4
	1000	553	23.2	5.5
	800	453	19.2	5.7
	600	350	15.1	5.8
	400	245	10.7	6.1
	200	138	6.1	6.9
	100	84	3.7	8.4

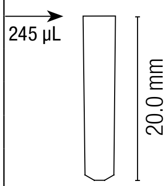
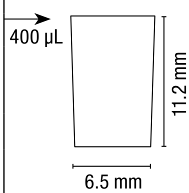
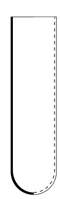
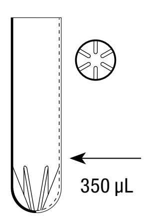
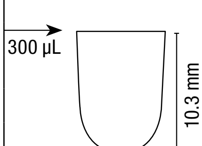
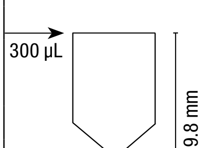
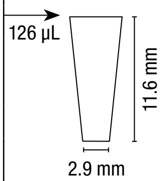
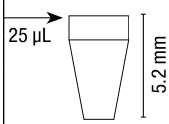
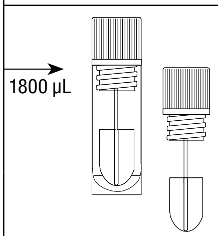
Product	Liquid volume, µL	Covered area, mm²	Liquid height, mm	Area/volume ratio, cm²/cm³
Nunc 384 DeepWell Plates				
	225	244	18.1	10.9
	200	220	16.4	11.0
	150	168	12.7	11.2
	125	142	10.8	11.4
	100	116	8.8	11.6
	50	62	4.7	12.4
	25	35	2.6	14
Nunc MicroWell Plates and Modules, F96, F16, F8				
	250	184	7.3	7.4
	200	154	5.9	7.7
	150	124	4.5	8.3
	125	109	3.8	8.7
	100	94	3.0	9.4
	75	79	2.3	10.5
	50	63	1.5	12.6
Nunc Immuno Tube 70 x 11				
	1500	760	27.0	5.1
	1000	520	18.4	5.2
	750	400	14.0	5.3
	500	280	9.5	5.4
	300	160	6.1	5.5
	250	140	5.6	5.6
	1	5	0.5	47.2
Nunc Immuno Tube 75 x 12 (StarTube 75 x 12)				
	1500	695 (815)	24.4 (25.5)	4.6 (5.4)
	1000	480 (600)	16.9 (18.0)	4.8 (6.0)
	500	260 (380)	9.3 (10.4)	5.2 (7.6)
	350	195 (315)	7.0 (7.6)	5.6 (9.0)
	300	175 (290)	6.2 (6.8)	5.8 (9.7)
	250	155 (260)	5.4 (5.8)	6.2 (10.4)
Nunc MicroWell Plates and Modules 0.30 mL, U96, U16, U8				
	250	175	8.7	7.0
	200	145	7.2	7.3
	150	115	5.7	7.7
	125	100	5.0	8.0
	100	85	4.2	8.5
	75	68	3.4	9.1
	50	52	2.6	10.4
Nunc MicroWell Plates 0.30 mL, V96				
	250	177	9.2	7.1
	200	147	7.8	7.3
	150	117	6.4	7.8
	125	101	5.6	8.1
	100	86	4.9	8.6
	75	71	4.1	9.4
	50	55	3.4	11.0

Table 1 (cont'd.)

Product	Liquid volume, µL	Covered area, mm²	Liquid height, mm	Area/volume ratio, cm²/cm³
Nunc 384 Well Plates				
	110	144	10.5	13.1
	100	133	9.7	13.2
	80	110	8.0	13.7
	60	86	6.3	14.3
	40	61	4.4	15.3
	20	36	2.3	17.6
	10	32	1.2	21.9
Nunc ShallowWell Plates				
	22.5	38.5	4.8	17.1
	20	35.5	4.5	17.6
	15	28.6	3.8	19.1
	12.5	25.2	3.4	20.2
	10	21.5	3	21.5
	5	13	1.9	26
	2.5	7.8	1.1	31.2
Product	**Volume in tubes, µL**	**Paddle area covered**	**Liquid height on paddle, mm**	**Area/volume cm²/cm³**
Nunc Immuno Stick				
	1000	520	8.5 (0.7)	5.2
	500	335	10.5 (0.7)	6.7
	250	176	6.2 (0.7)	7

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