Application Note

# Edge Effect in Thermo Scientific Nunc MicroWell ELISA

*Peter Esser, M.Sc., Senior Scientist, Thermo Fisher Scientific*

## Key Words

Thermo Scientific™ Nunc™ MicroWell™ plate, ELISA, edge effect, differences in optical density, temperature, illumination

## Goal

The goal of this application note is to describe the pinciples of the “edge effect” when adsorbing biomacro-molecules to polystyrene during ELISA.

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.

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To demonstrate a pronounced edge effect caused by temperature differences, a stack of 5 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 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.

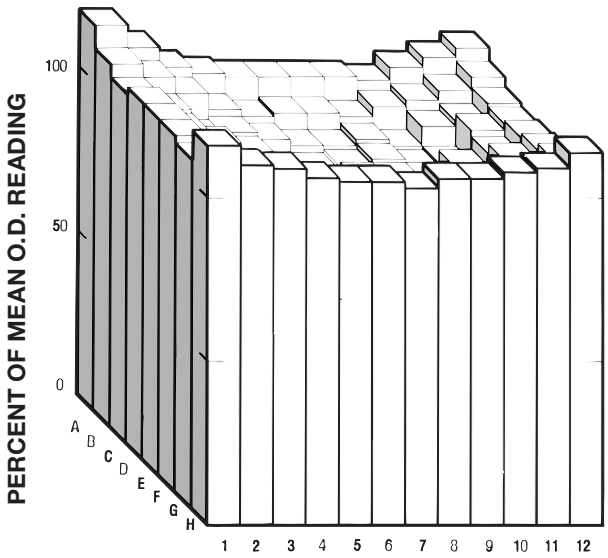


Fig. 1

Block diagram of the plate O.D. readings from $H^2O^2$/OPD substrate reactions in a Nunc 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.



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China Toll-free: 800-810-5118 or 400-650-5118; Singapore +65-6872-9718; Japan: +81-3-5826-1616; Korea +82-2-2023-0640;
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