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Tech Note

Enhanced Incubation Uniformity of BioTek's ELx808 Microplate Reader

Application to a Highly Temperature-Sensitive Endotoxin Assay

Temperature uniformity is a critical factor in microplate-based enzymatic assays. In kinetic measurements, even a small temperature variation across the microplate can produce unacceptable results. The incubator of Bio-Tek's EL_x808 microplate reader incorporates a zonal heating system with heating plates located both above and below the microplate. This system maintains extremely uniform and stable temperatures in the center as well as the edges of the microplate. For example, at an incubation temperature of 37°C, a microplate containing 200 µL of solution in each well displays a maximum well-to-well variation of less than 1°C , with an overall coefficient of variation of *less than 1%* [1].

To test the incubation qualities of the EL_x808 reader, an independent laboratory has recently carried out studies using a highly temperature-sensitive kinetic assay for the quantitative determination of endotoxins in solution. In this assay [2], Gram negative bacterial endotoxin catalyzes the activation of a proenzyme in Limulus Amebocyte Lysate (LAL), where the initial rate of this activation is determined by the endotoxin concentration. The activated enzyme releases *p*-nitroaniline (*p*NA) from a synthetic substrate, producing a yellow color which is measured at 405 nm continuously throughout the incubation period. The endotoxin concentration is typically determined from the amount of time required for the appearance of color (i.e., the time required for the enzymatic reaction to produce a specified change in absorbance at 405 nm).

In order to fully characterize the well-to-well variability of this assay when performed using the EL_x808 reader, 100 µL of a known endotoxin standard (concentration: 0.5 EU/mL) was pipetted into each well of a microplate. After a 10 minute pre-incubation [3], 100 µL of LAL/*p*NA reagent was added to each well. Throughout the succeeding incubation, the absorbance of each well was monitored at 405 nm at specified intervals. The time required for the enzymatic reaction to produce a change in absorbance of 0.200 at 405 nm was then determined for each well. The results for five such plates are summarized below.

Microplate Number	Mean Time for Absorbance Increase of 0.200 at 405 nm	% C.V. [4] (Entire Plate)
1	1139 sec.	2.42%
2	1145 sec.	2.21%
3	1145 sec.	2.83%
4	1172 sec.	1.84%
5	1208 sec.	1.80%

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The low values for the coefficient of variation for this temperature-sensitive assay clearly indicate that the EL_x808 provides extremely uniform temperature control across the entire microplate. It should also be noted that many incubating readers contain heating chambers which are completely separate from the optical components, and thus the microplate temperature is not controlled while absorbance measurements are carried out. The EL_x808, on the otherhand, maintains constant temperature control while reading, permitting more frequent kinetic measurements. This design also helps to prevent evaporation, as moist air is kept in the wells, enabling kinetic measurements over longer periods of time.

[1] Microplate temperature measurements were carried out as follows. A customized microplate (Nunc flat bottom) containing thermistors in 12 representative wells (A1, A5, A8, A12, D1, D5, D8, D12, H1, H5, H8, and H12) was filled with 200 μL of water per well. The temperature in each well was determined at equilibrium from thermistor resistance measurements.
[2] The LAL kit is manufactured by BioWhitakker, Inc.
[3] All incubations for the LAL study were at 37°C.
[4] The coefficient of variation (%C.V.) was determined using the mean and standard deviation values for all 96 wells of the microplate. For individual columns, the values ranged from 0.26 to 2.81%, and for individuals rows, the values ranged from 1.19 to 3.28%.

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