APPLICATION NOTE

Superior sensitivity from the EnVision Nexus multimode plate reader for luminescence detection technology.

Introduction

This application note demonstrates the luminescence detection capabilities of the newest Revvity multimode plate reader: the EnVision® Nexus™. The ATPlite™ 1step luminescence assay kit was used to evaluate signal and sensitivity measurements across the EnVision Nexus luminescence detection modes (enhanced and ultrasensitive).

The EnVision Nexus is a high-throughput multimode plate reader built for speed and sensitivity. As the next generation of Revvity's EnVision® platform, it continues to deliver the high sensitivity and optimal results expected from EnVision instruments. The EnVision Nexus is equipped with standard detection technologies to enable luminescence, absorbance, fluorescence intensity, fluorescence polarization, and time-resolved fluorescence measurements. It can also be equipped with optional technologies to enable Ultrasensitive Luminescence, Alpha, and TRF laser measurements, making the EnVision Nexus a high-performing instrument for all assay applications.

For research purposes only. Not for use in diagnostic procedures.

Figure 1: EnVision Nexus multimode plate reader.

The EnVision Nexus excels at luminescence detection and offers three detection modes – standard and enhanced, which are included with the base instrument, and an optional ultrasensitive (US) module. Standard luminescence is suitable for larger well sizes and bottom reading and can be combined with other readout technologies. The enhanced luminescence mode relies on an aperture within the standard beam path to reduce crosstalk and improve detection of samples with low signal; it is ideal for plates with high well densities (384- or 1536-well). The US mode uses a separate beam path to bring the detector closer to the sample and relies on a specialized aperture to block stray light, thereby boosting signal, decreasing crosstalk, and minimizing drift effects.

Methods

The newly launched EnVision Nexus multimode microplate reader (#HH36000002) was evaluated for its luminescence detection capabilities across two detection modes: 'enhanced' and 'ultrasensitive'. First, to assess the reading speed of the plate reader, a read time test was performed to determine the time required to measure an entire 384-well plate using each detection mode. Second, to evaluate the luminescence signal detection, data were generated using the ATPlite™ 1step luminescence assay kit (#6016736). The ATPlite 1step is a homogeneous, single-step luminescence assay for the quantification of viable cells. Designed as an alternative to colorimetric, fluorometric and radioisotopic assays, it evaluates the proliferation and cytotoxicity of cultured mammalian cells based on the production of light resulting from the reaction of ATP with added firefly *(Photinus pyralis)* luciferase. ATP serves as a marker for cell viability due to its presence in all metabolically active cells. The concentration of ATP declines rapidly when cells undergo necrosis or apoptosis, thus monitoring ATP levels is a good indicator of cytotoxic, cytostatic and proliferation effects.

In preparation for the assay, THP1 cells were cultured to competency. Cells were then 2-fold serially diluted to cell densities ranging from 550,000 to 17 cells per well. The ATPlite assay was run following the one-step protocol outlined in the kit TDS. Briefly, 25 µL of each cell density was plated in a gray 384-well AlphaPlate (#6005350) before an equal volume of prepared ATPlite reagent (25 µL) was added to each well. A set of background wells (containing no THP1 cells) were also included on the plate. An identical setup was prepared on a black 384-well plate (#6007270) for comparison to the readout from gray plates. Following the addition of the ATPlite reagent, plates were mixed thoroughly for 10 minutes then measured in enhanced and ultrasensitive modes. Assay data were plotted as Signal to Background (S/B). The LLOQ (lower limited of quantitation) was calculated for each reading mode by taking the average signal counts of the background wells (no THP1 cells) added to a measure of five times the standard deviation of the background wells (LLOQ = avg. background signal (counts) + (5 x $SD_{background}$)), given as a concentration of cells/well.

Results and discussion

Read Time Test

A 'total read time' measurement was calculated for each detection mode which encompassed the time required for the plate to enter the instrument (Entry Time); time required to read the plate wells (Reading Time), and time for the plate to exit the instrument post-reading (Offload Time). The Reading Time for a 384-well plate was 01:24 (mm:ss) in enhanced detection mode. Running the EnVision Nexus in ultrasensitive mode did not significantly increase Reading Time (01:26), and Total Read Time was similar between detection modes: 01:38 for enhanced compared to 01:49 for US mode. These reading times highlight that the improved sensitivity benefits of the EnVision Nexus ultrasensitive mode can be applied without major impact to throughput.

Table 1: Plate reading time data for a full 384-well plate on the EnVision Nexus using the enhanced and Ultrasensitive Luminescence detection modes. The 'Total Read Time' measurement incorporates the time for the plate to enter the instrument (Entry Time); time to read all plate wells (Reading Time), and time for the read plate to exit the instrument (Offload Time).

Detection Mode	Entry Time (mm:ss)	Reading Time (mm:ss)	Offload Time (mm:ss)	Total Read Time (mm:ss)
Enhanced	00:11	01:24	00:03	01:38
Ultrasensitive	00:20	01:26	00:03	01:49

ATPlite Assay

The EnVision Nexus successfully measured ATP across a broad range of cell dilutions in both enhanced and ultrasensitive detection modes (Figure 2). When using the gray, 384-well AlphaPlates the S/B was on average 2.2 times higher in US mode compared to enhanced mode of the EnVision Nexus, highlighting the improved detection capabilities of this mode. The overall sensitivity, calculated as the LLOQ, was over three times greater in US mode (LLOQ = 0.94 cells/well) compared to the enhanced mode (LLOQ = 3.83 cells/well; Table 2). Importantly, this demonstrates that US mode can be employed to improve sensitivity without significantly impacting throughput, since ultrasensitive and enhanced mode required similar total read time.

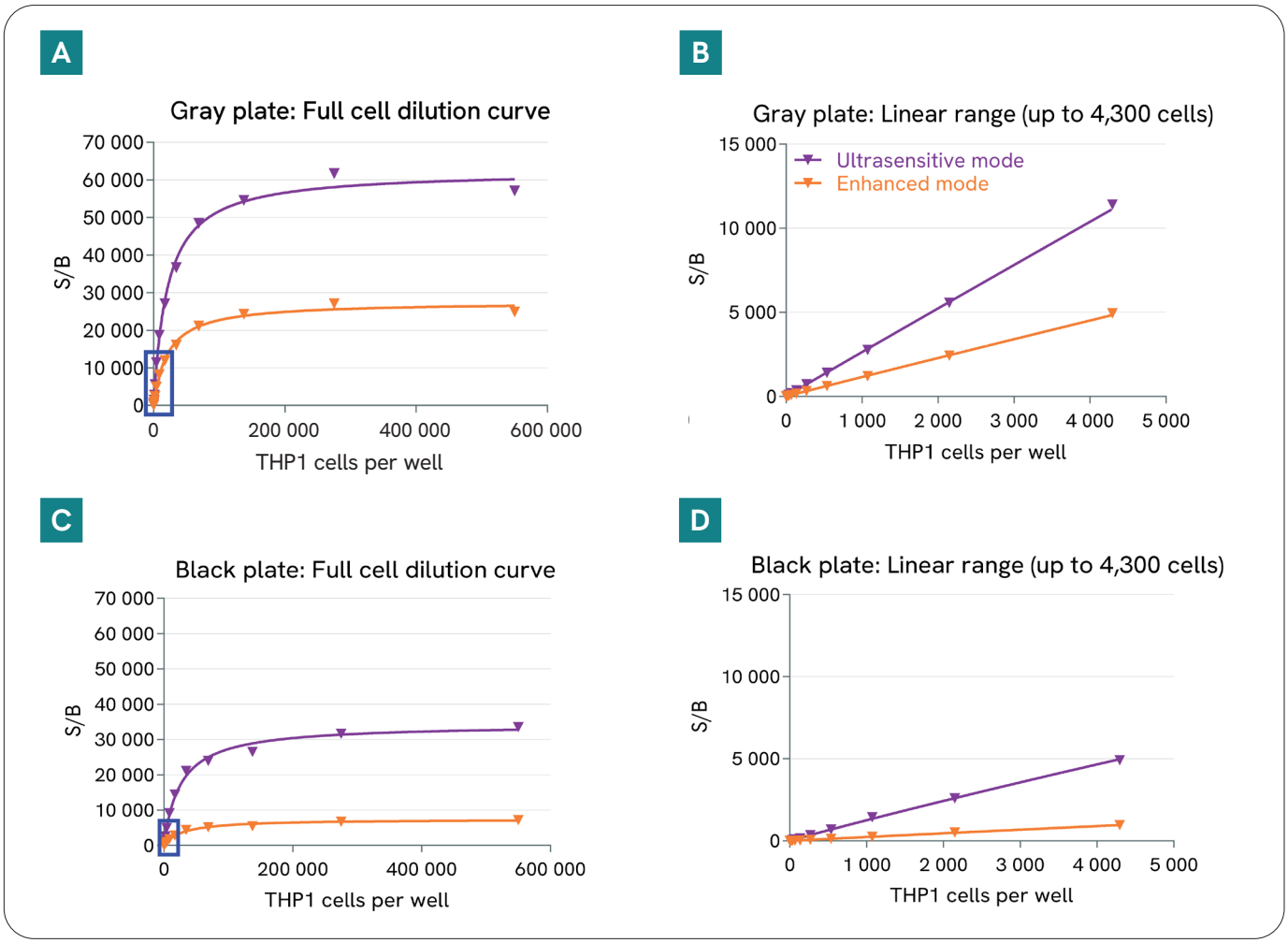

Figure 2: S/B plots of the ATPlite 1step Luminescence assay results from THP1 cell dilutions. Top: S/B plots for luminescence measurements using gray AlphaPlates. Panel A depicts the complete dilution curve (0 – 550,000 cells/well) and Panel B shows the blue boxed detail of the linear range of the assay (up to 4,300 cells/well). Bottom: S/B plots for luminescence measurements using black plates. Panel C depicts the entire dilution curve (0 – 550,000 cells/well) and Panel D shows the blue boxed detail of the linear range of the assay (up to 4,300 cells/well).

Table 2: LLOQ (cells/well) values for the enhanced and Ultrasensitive Luminescence detection modes using gray and black 384-well plates.

Detection Mode	LLOQ (cells/well)—gray plate	LLOQ (cells/well)—black plate
Enhanced	3.83	15.72
Ultrasensitive	0.94	5.13

The luminescence signal was decreased when using the black, 384-well plates in both the enhanced and ultrasensitive detection modes of the EnVision Nexus (Figure 3C & 3D). Similar to the results seen on gray plates, ultrasensitive mode produced the highest S/B measurements which were, on average, 5.0 times higher than enhanced mode. The US mode also showed an improved LLOQ (5.13 cells/well), relative to enhanced mode (LLOQ = 15.72 cells/well; Table 2). The reduced signal measured from black-colored plates is expected, as black plates "quench" the signal by absorbing some of the light produced by the assay. Gray plates provide an intermediate signal level of quenching relative to white plates, which provide the highest overall signal measurements. Luminescence detection for the EnVision Nexus was optimized using gray plates. Gray colored plates may benefit some luminescence assays by reducing background while maintaining high signal. However, for applications that produce an especially bright luminescence signal, black plates may be helpful in reducing well-to-well crosstalk.

Conclusions

The EnVision Nexus displayed strong performance of its luminescence detection technology. The built-in enhanced detection mode performed well across a broad range of cell dilutions and demonstrated good sensitivity. The optional, add-on ultrasensitive mode more than tripled sensitivity without requiring a significant increase in reading time. As expected, the luminescence signal was impacted by plate color, and the plate selected for use with luminescence assays will need to balance the need for crosstalk reduction versus sensitivity. Overall, the EnVision Nexus features best-in-class speed and sensitivity making it well-suited for applications requiring high-throughput luminescence detection.

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