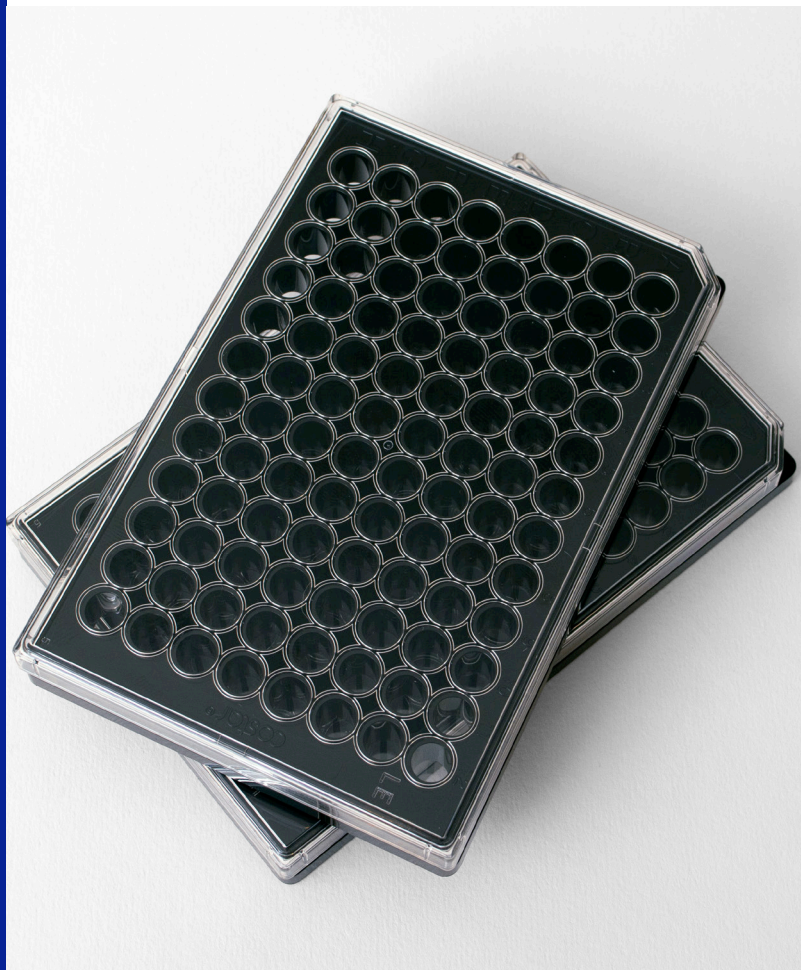
Making the Medicines of Tomorrow

Lonza

Improve your PyroGene® Assay performance with the 96-well clear bottom black microplates

At low endotoxin levels, even subtle variations in background fluorescence can affect your results. Selecting the right plate helps you minimize background producing more precise, high-quality data.

This application note describes the initial evaluation of the 96-well clear bottom black microplates (catalog no. 00317940) and a subsequent study comparing these plates with the clear plate (catalog no. 25-340). The results demonstrate that the 96-well clear bottom black microplates are suitable for the PyroGene® Assay and provide better signal-to-noise ratio, better fluorescence detection sensitivity, and more consistent results than clear plates — all without changing your workflow.



Initial qualification

Initial evaluation of the 96-well clear bottom black microplates consisted of endotoxin contamination testing, inhibitory well testing and Initial Qualification (IQ) testing with three lots of plates using the PyroGene® Assay and the PyroWave® XM Reader. Additionally, IQ assays were also performed with the same three plate lots using the Nebula® Multimode Reader.

Comparative study experimental overview

This study consisted of two parts:

1. Background fluorescence analysis
2. Variations in delta Relative Fluorescence Units (ΔRFU) measured in the blank

Part 1:

Background fluorescence analysis

To demonstrate the advantages of using the 96-well clear bottom black microplates compared to the clear plate (25-340), three plate lots of each plate type were tested. The fluorescence of the empty wells was then measured using the PyroWave® XM Fluorescence Reader.

Results

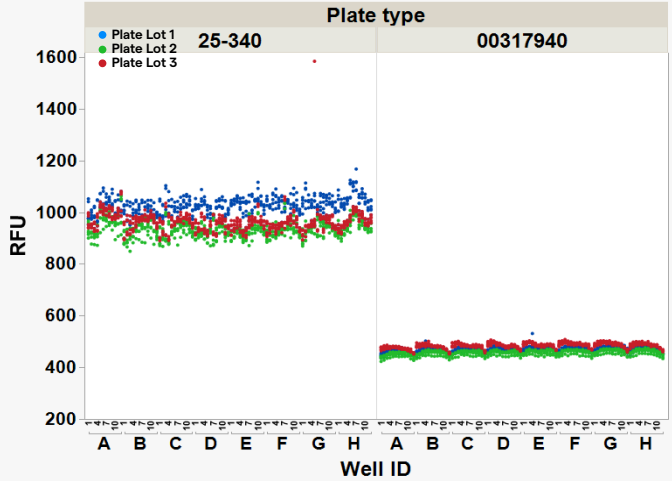
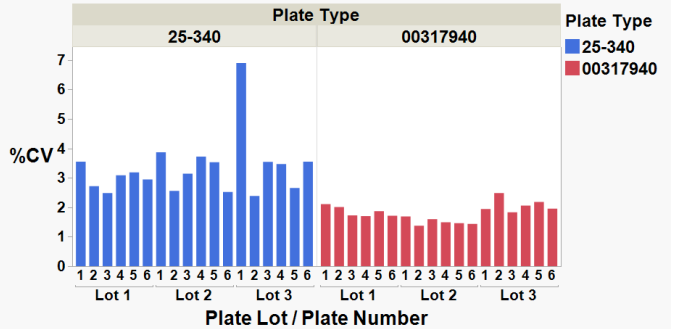


Figure 1.
Comparison of background fluorescence signal from empty plates (lots 1, 2, and 3) measured at ex/em 380/440 nm.

The background fluorescence signal was higher in clear plates (25-340) compared to 96-well clear bottom black microplates (00317940). 96-well clear bottom black microplates reduce light reflection, autofluorescence, and well-to-well light scattering, resulting in lower background noise and improved signal-to-noise ratio – critical for detecting low-level fluorescence and enhancing fluorescence detection sensitivity.

96-well clear bottom black microplates demonstrated lower well-to-well variability compared to clear plates, resulting in higher precision. They also showed reduced lot-to-lot variability, leading to more consistent results across plate lots.



	Clear plate (25-340)	96-well clear bottom black microplates (00317940)
Average %CV	3.321	1.812
Range	2.4% – 6.9%	1.4% – 2.5%

Figure 2.
The percentage coefficient of variation (%CV) of RFU values for 25-340 clear plates and 00317940 96-well clear bottom black microplates.

The 96-well clear bottom black microplates exhibited lower %CV and less variation, indicating higher consistency in data compared to clear plates.

Part 2:

Variations in delta Relative Fluorescence Units (ΔRFU)

Evaluation of blank wells is important as it would reflect the background signal for the assay and variations in the blank can lead to inconsistent and inaccurate results. Blank wells containing LAL Reagent Water (LRW) and PyroGene® Reagent mix were evaluated for ΔRFU (the difference in between initial RFU value and RFU value after 1 hour) in a fluorescence uniformity assay. As in Part 1, three lots of each plate type were evaluated using one lot of PyroGene® Reagents. This ensures that any observed variability in ΔRFU is attributable to plate type rather than reagent lot differences.

Results

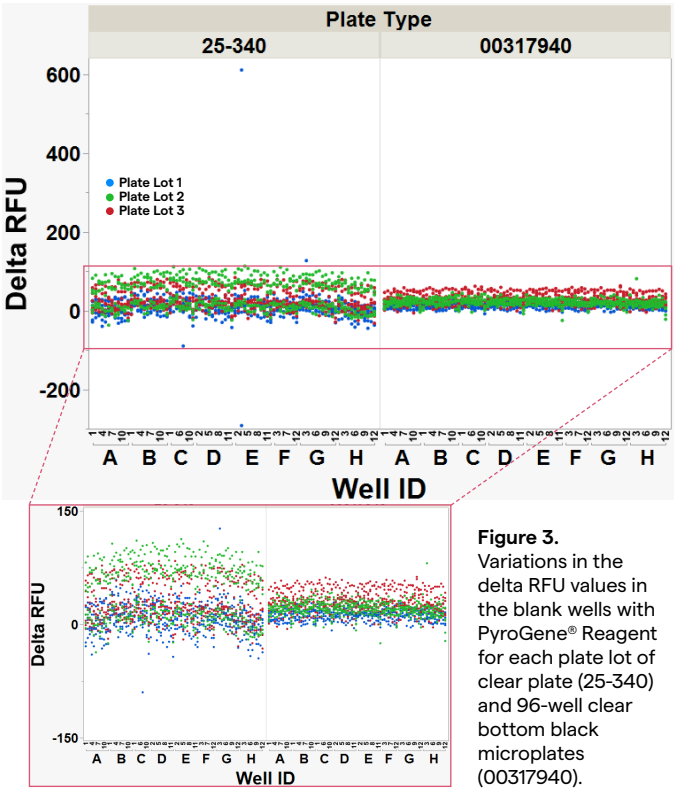


Figure 3. Variations in the delta RFU values in the blank wells with PyroGene® Reagent for each plate lot of clear plate (25-340) and 96-well clear bottom black microplates (00317940).

96-well clear bottom black microplates (00317940) exhibited lower well-to-well and plate-to-plate variability in delta RFU values compared to the clear plates (25-340). Additionally, lot-to-lot variability was less in the 96-well clear bottom black microplates, decreasing the risk of inaccurate data across different plate lots.

Why switch to the 96-well clear bottom black microplates for PyroGene® Assays?

1. **Improved signal-to-noise clarity**
   This plate reduces the background fluorescence, thereby increasing the signal-to-noise ratio and enhancing fluorescent detection sensitivity. This helps you differentiate low-level positives from noise — which is especially critical for samples near the detection limit.
2. **Consistent, reproducible results**
   Lower background variability across wells helps improve data consistency and reproducibility, which is critical for sensitive assays like the PyroGene® rFC Assay.
3. **Seamless fit with existing workflows**
   Switching plates does not mean changing your process. The 96-well clear bottom black microplates are fully compatible with the PyroGene® rFC Assay and Lonza readers (i.e. PyroWave® XM Fluorescence Reader and Nebula® Multimode Reader). No additional validation steps are required.

In short, switching to 96-well clear bottom black microplates is a low-effort, high-impact improvement to boost your PyroGene® rFC Assay performance.

Ready to adopt

Per major pharmacopeia, "If employing plastic apparatus, such as microplates and pipette tips for automatic pipettors, use apparatus that is shown to be free of detectable endotoxin and does not interfere in the test." This testing has been done for you for each lot of 00317940. Every lot of plates is tested for endotoxin content and inhibition prior to release. Additionally, each lot of plates is subject to functionality testing. This is performed by running an IQ assay with PyroGene® Reagents. Results for all these tests are found on the lot-specific Certificate of Analysis. Customers wanting to do confirmatory testing in-house, may choose to repeat the IQ assay to demonstrate acceptable standard curve performance.

Realize the advantages of utilizing the 96-well clear bottom black microplates (00317940) and upgrade your workflow now.

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