TECHNICAL NOTE

Excellent AlphaLISA assay performance using the EnVision Nexus.

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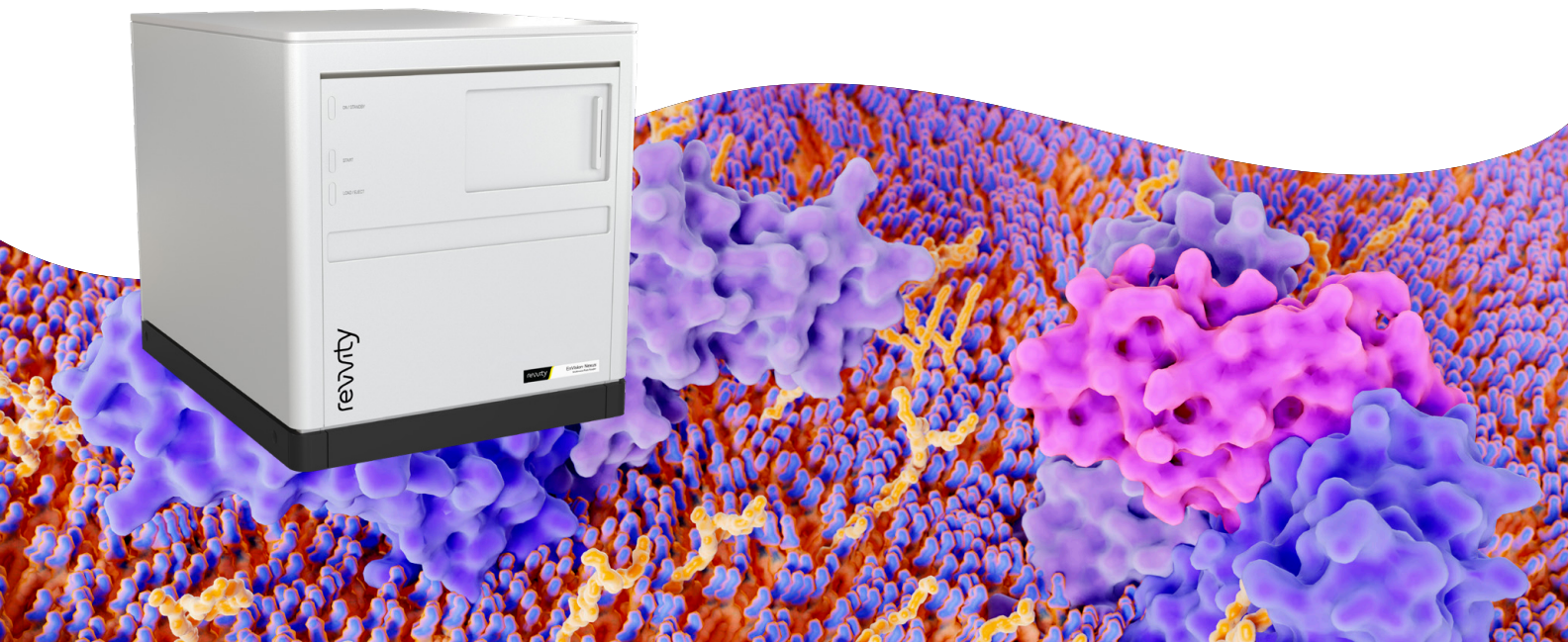
Highlights

- Excellent assay performance
- High throughput
- High sensitivity
- Minimized crosstalk for all Alpha technologies

Introduction

In life sciences and biomedical research, detection and monitoring of protein expression, activity and interaction are key to understand cellular processes and for developing potential new treatments. AlphaLISA™ and AlphaScreen™ are bead-based assay technologies used to investigate these interactions and properties. They combine a no-wash and no-separation approach with high sensitivity and a large dynamic range, and are compatible with many sample types including serum, plasma and cell lysates. The EnVision Nexus™ multimode plate reader offers two options for Alpha detection: enhanced (ENH) and high-throughput screening (HTS). Both Alpha options deliver high-quality data using apertures that block stray light from adjacent wells to minimize crosstalk. However, the Alpha (HTS) option is significantly faster due to its optical design which is optimized for speed. While Alpha (HTS) is limited to single channel measurements, Alpha (ENH) can also be used for AlphaPlex™ measurements.

In this tech note, we evaluate the assay performance of EnVision Nexus compared to EnVision™ 2105 using an AlphaLISA Interleukin 8 assay.



Materials and methods

AlphaLISA Human Interleukin 8 (IL8) Kit

The AlphaLISA Human Interleukin 8 (IL8) Kit (Product No.: AL224C) was used. For best homogeneity all incubation steps for the different sample types were performed as mastermixes in reaction tubes. A dilution series from 100,000 pg/mL to 0.3 pg/mL was created according to the assay manual in triplicates. For the Z′ calculations 30 replicates of Assay Background (0 pg/mL), Standard 4 (3,000 pg/mL) and Standard 9 (10 pg/mL) were added to the plate layout.

Preparation of 384-well plates

Mastermixes were pipetted into a StorPlate-384 deepwell, V-bottom (Part Number: 6008690) during the last incubation step. To minimize pipetting errors, an automated liquid handling workstation was used to pipette 20 µl from the StorPlate-384 into the wells of a 384-well AlphaPlate™ (Part Number: 6005359). Plates were sealed with TopSeal A, black (Part Number: 6050173) which was removed before the start of the measurement.

Measurements

Measurements were conducted immediately after the end of the last incubation step. Alpha measurements on the EnVision Nexus or EnVision™ 2105 were performed either with the Alpha (ENH/STD) or the Alpha (HTS) option, as specified in Table 1. For high-precision settings we used an excitation time of 180 ms and a detection time of 370 ms (550 ms in total). High-throughput settings utilized a 35 ms excitation time and a 65 ms detection time (100 ms in total).

Table 1: Settings used for data acquisition.

	EnVision Nexus		EnVision 2105	
Technology	Alpha (ENH)	Alpha (HTS)	Alpha (STD)	Alpha (HTS)
Aperture	ELD	ULD	-	A384
Filter module	AlphaScreen 5001	-	AlphaScreen emission 570	-
Excitation source	680 nm laser	680 nm laser	680 nm laser	680 nm laser
Emission filter	575/110 nm	650 nm shortpass	570/100 nm	650 nm shortpass
Crosstalk minimization reading mode	On	On	-	-
Measurement duration (start to end) 384-well plate	4min 31s (1min 34s for HTS settings)	1min 36s	4min 33s	1min 41s
Ambient temperature	On	On	-	-

Calculations and data analysis

Revvity Signals™ was utilized for data analysis.

For the IL-8 dilution series, we employed a four-parameter logistic (4PL) regression fit with 1/Y data weighting. The concentrations beyond the hook point were removed.

The lower detection limit (LDL) was calculated by interpolating the average background counts (µ) of 12 wells (Assay Background) plus three times the standard deviation (σ) of the background on the standard curve.

Z′ calculations:

$$Z' = \frac{3\,\sigma\,(\text{High Standard}) - 3\,\sigma\,(\text{Assay Background})}{\mu\,(\text{High Standard}) - \mu\,(\text{Assay Background})}$$

High Standard = Standard 4 (large assay window) or Standard 9 (narrow assay window)

Abbrevations

4PL	Four-parameter logistic
Alpha	Amplified Luminescent Proximity Homogeneous Assay
ENH	Enhanced
HTS	High-throughput screening
LDL	Lower detection limit
STD	Standard

Results

1. The EnVision Nexus Alpha technologies show unprecedented assay performance

The performance of an assay is not only limited by the instrument performance but also by assay specific factors such as the underlying biology, antibody affinity and experimental limitations (e.g. pipetting precision and well to well differences of cultured cells). Here, we chose the AlphaLISA Interleukin 8 (IL-8) assay as an example for an immunoassay and used the provided standard to calculate EC_{50} and lower detection limit (LDL). Measurements were performed with Alpha (ENH) and Alpha (HTS) options on an EnVision Nexus and as comparison on its predecessor EnVision 2105 using one instrument equipped with an Alpha (STD) module and another equipped with the Alpha (HTS) module (see Figure 1). For all 4 measurements, EC_{50} values only varied slightly in the expected range between different experimental preparations.

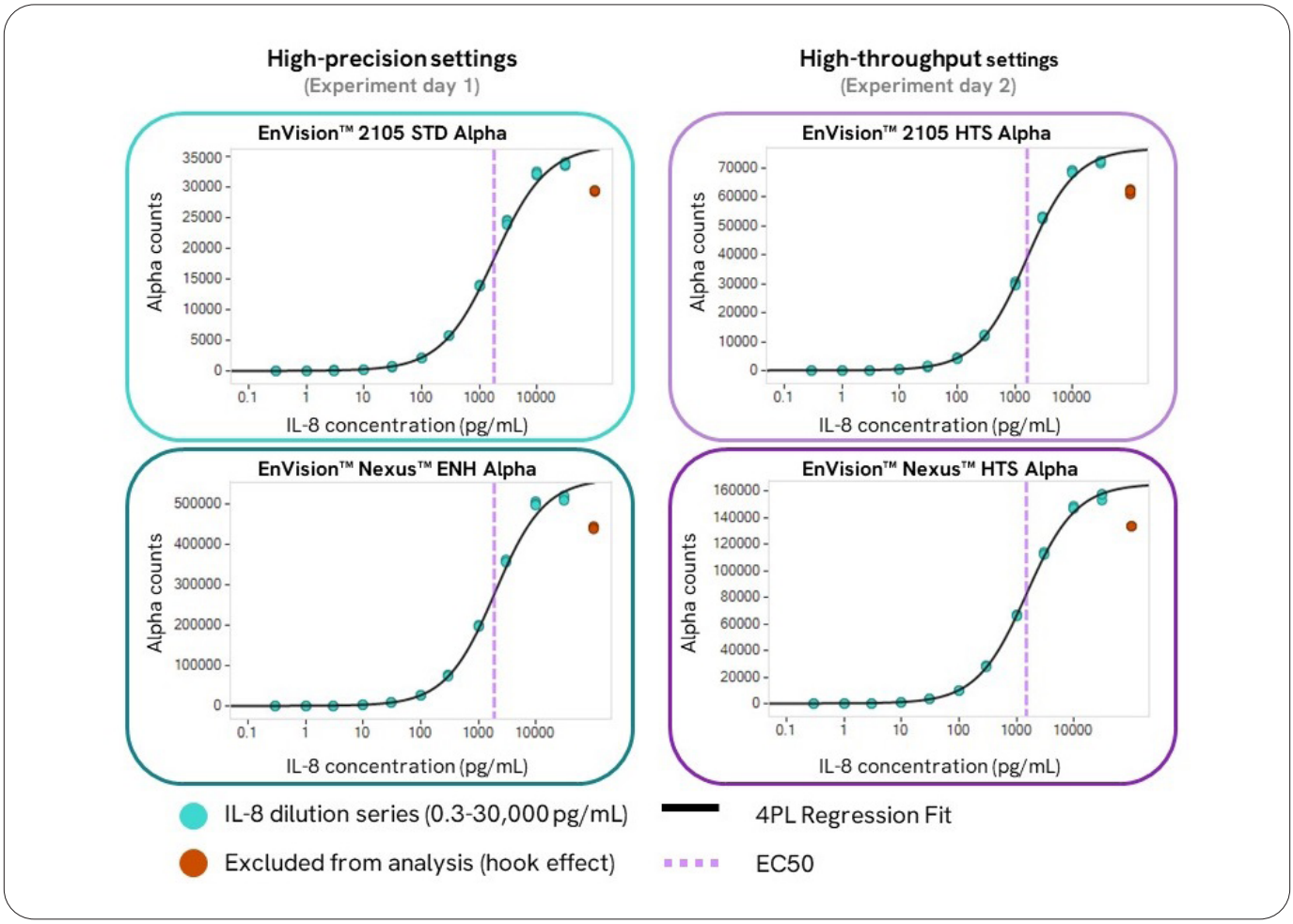


Figure 1: Dose-response curves for the AlphaLISA IL-8 assay on the EnVision Nexus and the EnVision 2105 The IL-8 standard sample was diluted according to the assay manual. The data were acquired with the default settings of the respective technologies. A four-parameter logistic (4PL) regression fit was applied to calculate the lower detection limit (LDL) and the half-maximal effective concentration (EC_{50}). The 100,000 pg/mL IL-8 concentration was excluded from the analysis due to a signal drop which indicates a saturation of the binding (hook effect). Experiments for Alpha (STD) and Alpha (ENH) were conducted on a different day than those for Alpha (HTS), and the respective standard dilutions had to be prepared immediately before each experiment. However, EC_{50} values matched within the expected range (Day 1: 2103 pg/mL (EnVision 2015 STD Alpha) and 1915 pg/mL (EnVision Nexus ENH Alpha); Day 2: 1598 pg/mL (EnVision 2105 HTS Alpha) and 1500 pg/mL (EnVision Nexus HTS Alpha)).

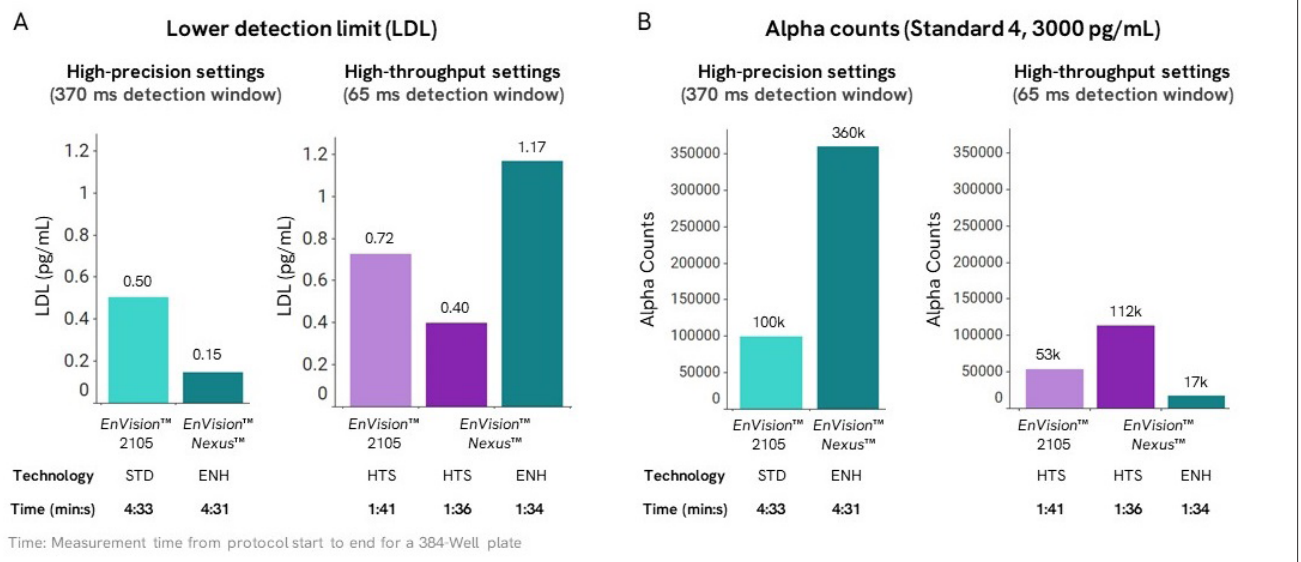


Figure 2: Alpha Counts and Lower Detection Limit Comparison A: Comparison of the lower detection limits between high-precision and high-throughput settings for the different measurement modes on the EnVision Nexus and the EnVision 2105. B: Alpha counts were measured for different measurement modes and detection windows. High-precision settings utilized 180 ms excitation with the Alpha laser and 370 ms detection. For high-throughput settings only 35 ms of excitation and 65 ms detection were used.

2. The EnVision Nexus Alpha (HTS) technology shows high performance with 3-fold higher throughput

We could demonstrate the significant improvements through the new optics of the EnVision Nexus, particularly when comparing the new Alpha (ENH) technology with the EnVision 2105 Alpha (STD) technology. The LDL achieved with Alpha (ENH) was 0.15 pg/mL, which is more than 3 times better than the LDL achieved with Alpha (STD) (see Figure 2A).

Using the Alpha high-throughput settings significantly reduces the total measurement time by a factor of 3 (from approximately 4 minutes and 30 seconds to only about 1minute and 30s for a 384-well plate). To achieve this speed advantage, the measurement time (excitation and emission) is reduced, resulting in a lower Alpha signal. When the Alpha (ENH) option is used with high-throughput settings, the counts decrease by a factor of ~20 (see Figure 2B). The Alpha (HTS) option was specifically designed to maintain high Alpha counts even under high-throughput conditions. By positioning the detector directly above the plate, it can capture more photons emitted from the Alpha sample. With high-throughput settings, this results in Alpha counts more than 6-times higher than with the Alpha (ENH) option. The shorter measurement time also has an impact on important statistical measures such as the LDL. The EnVision Nexus Alpha (HTS) option still achieved an LDL of 0.4 pg/mL, compared to an LDL of 1.2 pg/mL with the Alpha (ENH) option using high-throughput settings, and an LDL of 0.7 pg/mL on the EnVision 2105 with the Alpha (HTS) option (see Figure 2A).

3. Excellent Z′ values on the EnVision Nexus even for narrow assay windows

In our analysis of Z′ values, we found that for large assay windows from 0 to 3000 pg/mL, all Alpha options demonstrated robust performance with Z′ values of 0.95 or higher (Figure 3A). To challenge our instruments, we also calculated the Z′ value assuming an extremely narrow assay window, ranging only from 0 to 10 pg/mL (see Figure 3B). Under these conditions, the Alpha (ENH) mode on the EnVision Nexus with high-precision settings showed the best performance due to the high signal counts and low standard deviation, resulting in a Z′ value of 0.89 (Figure 3B). Despite lower signal counts when using Alpha (ENH) with high-throughput settings, the Z′ value of 0.65 indicated decent performance under these conditions. However, for optimal performance in a high-throughput scenario, Alpha (HTS) is the recommended option on the EnVision Nexus with a Z′ value of 0.76 (Figure 4).

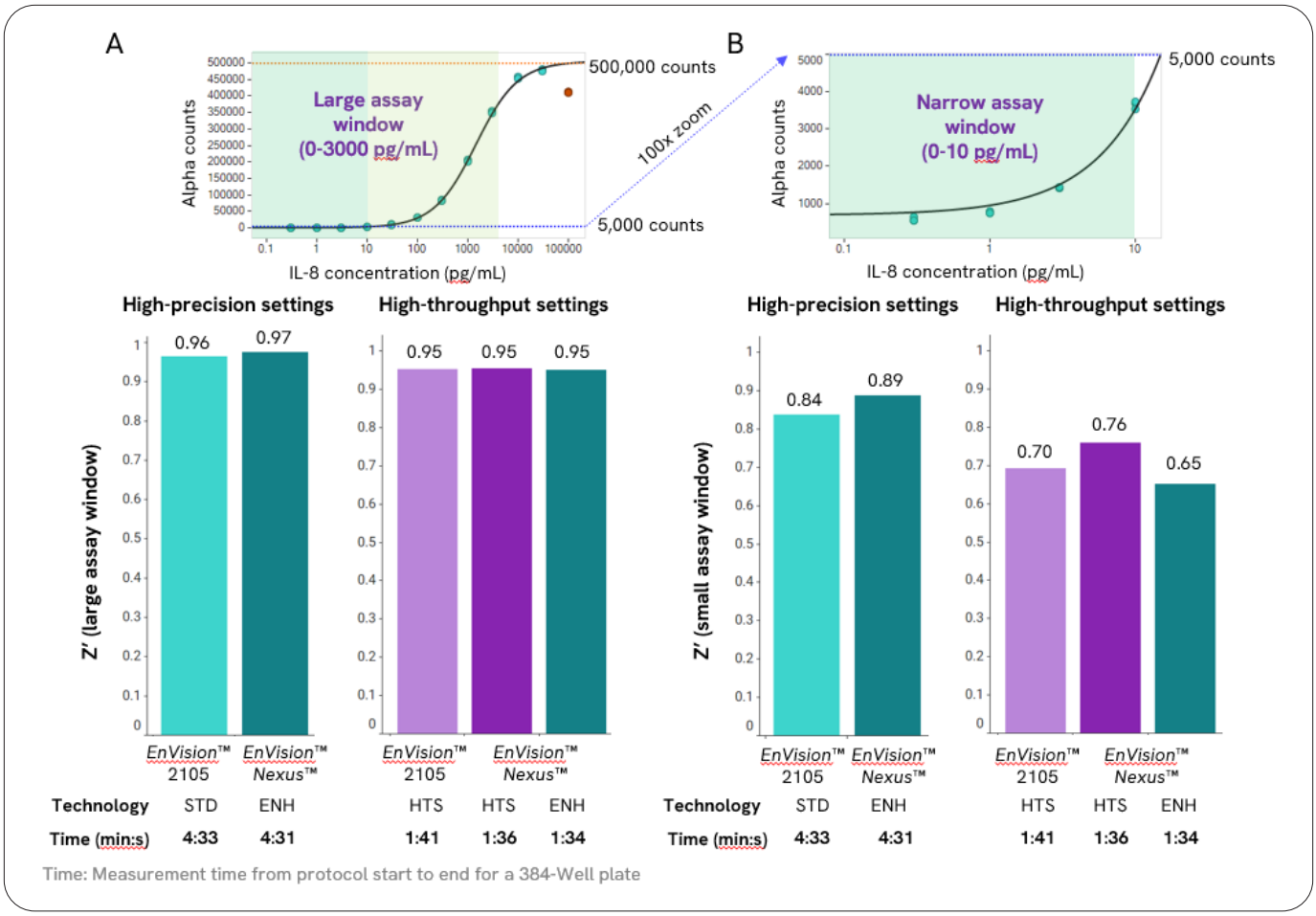


Figure 3: Z′ values calculated for Large and Narrow assay windows Z′ values were determined for both a large assay window (A) and for a small assay window (B). Each plate included 30 replicates of Assay Background samples (0 pg/mL IL8) , Standard 4 samples (3000 pg/mL IL8) for the large assay window (A), and Standard 9 samples (10 pg/mL IL8) for the narrow assay window (B). Measurements were performed using both high-precision and high-throughput settings on the EnVision 2105 and EnVision Nexus, using the different measurement modes.

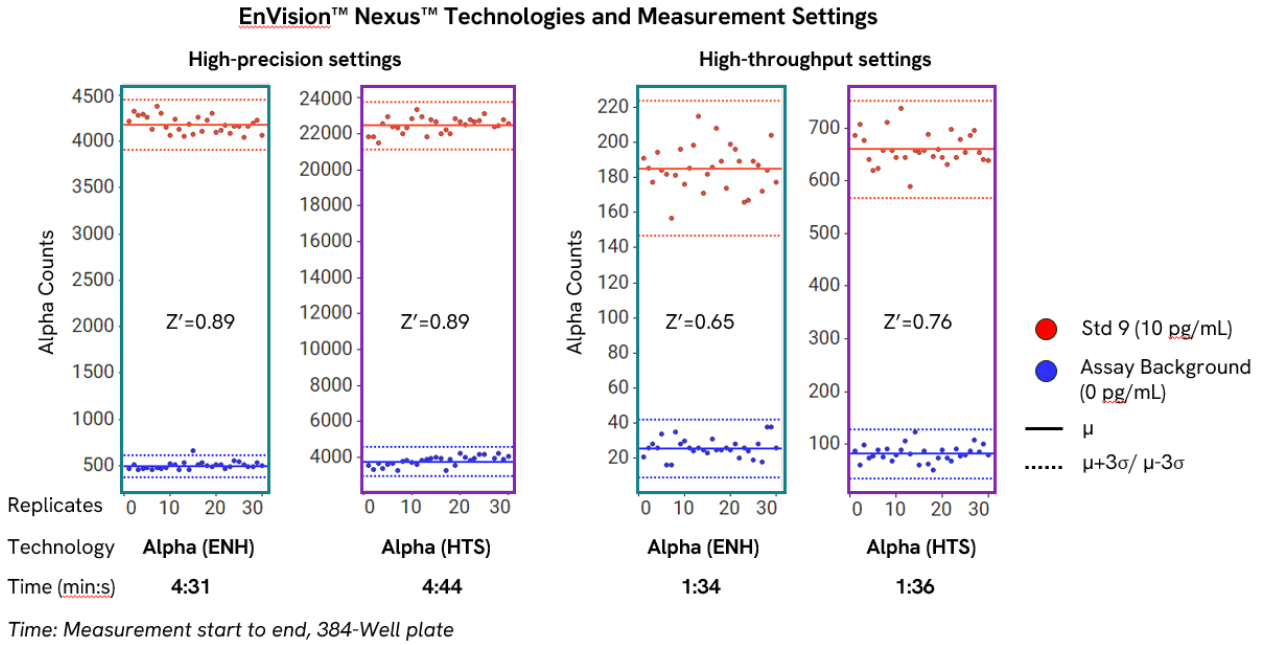


Figure 4: Optimal Configuration Based on Application Type These plots display raw data for four different measurement configurations on the EnVision Nexus: Alpha (ENH) and Alpha (HTS) either used with high-precision or high-throughput settings. The X-axis of each plot represents 30 replicates for Assay Background (0 pg/mL) and Standard 9 (10 pg/mL). The graph illustrates the variance and Z′ values for a narrow assay window.

Conclusion

The optical design of the EnVision Nexus allows for excellent Alpha performance. Especially the Alpha (ENH) option is superior to Alpha (STD) on other devices and complements the Alpha (HTS) option. For screening applications where high-throughput capability is essential the HTS Alpha option excels providing data of high quality almost 3-times faster than Alpha (ENH) measurements (see Figure 4).

If speed is not the decisive factor, Alpha (ENH) presents a more versatile option with several filter modules available for single-channel measurements as well as multiplexing applications using the available AlphaPlex™ filter modules (AlphaPlex Eu/Tb, Eu/Sm and Sm/Tb). For maximum flexibility, the EnVision Nexus can be equipped with both Alpha options.