

# High-performance Alpha technology on VICTOR Nivo.

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Alpha technology is a homogenous bead-based proximity assay with a solution for just about every research application. When Alpha Donor and Acceptor beads are brought together, excitation of the Donor beads at 680 nm triggers the release of a singlet oxygen molecule that causes a cascade of energy transfer in the nearby Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 1). The amount of analyte in the sample is proportional to the emission signal. Alpha technology is highly versatile as beads can be coated with various biomolecules, enabling detection of unique biological events.

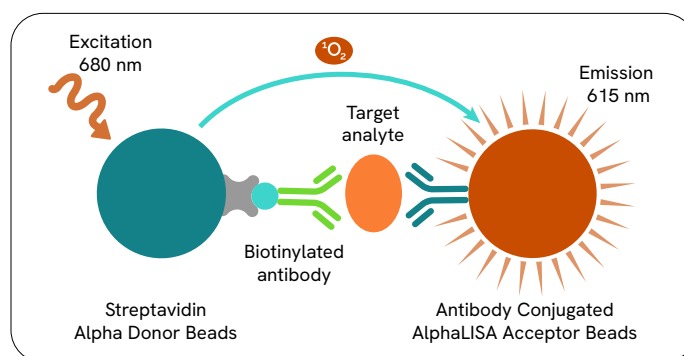
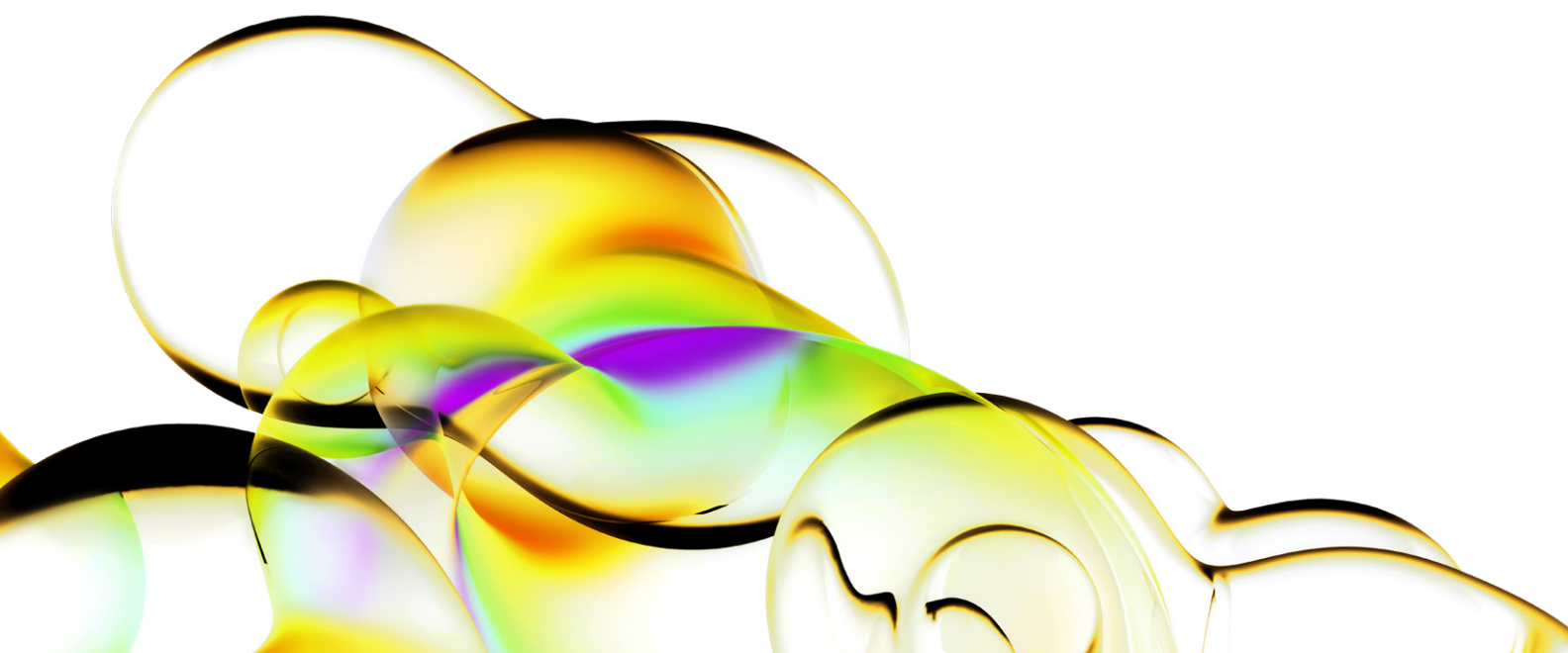


Figure 1: Assay principle for AlphaLISA biomarker detection assay (sandwich immunoassay format).

To bring this technology to research environments with lower budget capabilities, the VICTOR® Nivo™ multimode plate reader is now available with all optical components for high



Alpha performance technology: 680 nm diode Laser for Alpha Donor bead excitation and dedicated Alpha optics, consisting of a dichroic mirror (D660 nm) and Alpha emission filter (575/110 nm). The Alpha-enabled VICTOR Nivo comes with optimized measurement quality for Alpha-based assay technologies. This is now possible due to a new technical feature which minimizes background emissions by blocking the emission light path during the laser excitation.

To qualify the Alpha-enabled VICTOR Nivo on the basis of critical assay parameters, we have tested two AlphaLISA® assays, human insulin (Revvity, #AL204C) and p24 (human HIV) (Revvity, #AL207C). For comparison, both assays were run on the EnSight™ multimode plate reader with HTS Alpha module and standard protocol settings.

The human insulin AlphaLISA kit is widely used and is designed for the quantitative determination of human insulin in serum, buffered solution or cell culture. Insulin is a key player in the control of both carbohydrate and lipid metabolism and has been implicated in various diseases including diabetes, heart disease and obesity. The human immunodeficiency virus type-1 p24 protein (HIV p24) AlphaLISA detection kit is frequently used for HIV detection in blood, serum samples, and other bodily fluids in acute HIV seroconversion, in neonatal infection, and for monitoring of responses to antiviral drug therapy.

## Material and methods

AlphaLISA assays were performed with 5 µL sample in a final volume of 50 µL in Revvity's 96-well ½ Area AlphaPlates™ (#6002350). Standard dilutions (36 wells, each done in triplicate) were prepared according to the kit instructions, together with HIGH and LOW controls (x24 for each) containing the highest analyte concentration or no analyte, respectively. All pipetting steps were performed under green filtered laboratory lighting. Plates were then sealed with Black TopSeal-A (#6050173) and centrifuged for < 10 sec at low RPM (< 500) before measurement. Control parameters for the Alpha-enabled VICTOR Nivo were set according to Table 1.

Assay parameters were calculated from the results with MyAssay Desktop data analysis software (MyAssays Ltd. 2019, Version 4.2.15.727) by plotting a four-parameter logistics (4PL) fit curve through the standard dilutions data points (average value of triplets) using 1/Y<sup>2</sup> data weighting.

Lower limit of detection (LLD) was defined by interpolating 3x SD of the LOW controls from the baseline (fit coefficient a) on the standard curve. Concentration values refer to the final analyte dilution in the assay well. Additionally, Z'-value and dynamic range were determined from HIGH and LOW controls.<sup>1</sup>

Table 1: Alpha-enabled VICTOR Nivo parameters for Alpha measurements.

Z-Focus	12 mm
Emission spot size	4 mm
Excitation time	150 ms
Emission time	600 ms
Relative laser power	100 %
Measurement order	Left to right by rows

## Results

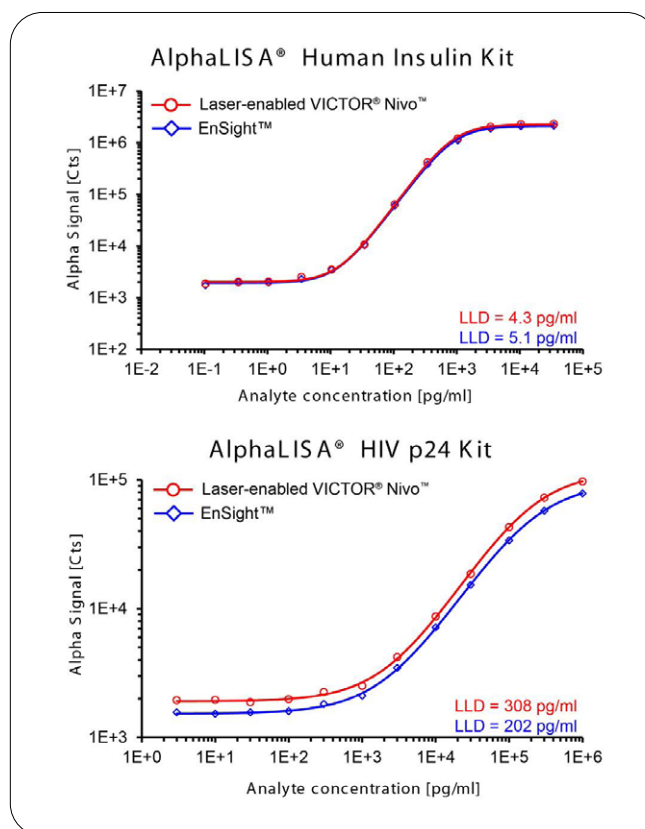


Figure 2: Comparison of AlphaLISA human insulin and HIV p24 assay performance between the Alpha-enabled VICTOR Nivo multimode plate reader and the EnSight equipped with HTS Alpha module. Assay performance and sensitivity (LLD) parameters were calculated with MyAssays Desktop data analysis tool.

Table 2: Assay performance parameters of the Alpha-enabled VICTOR Nivo in comparison to the EnSight multimode plate reader equipped with HTS Alpha module.

	AlphaLISA human insulin kit		AlphaLISA HIV p24 kit	
	Alpha-enabled VICTOR Nivo	EnSight	Alpha-enabled VICTOR Nivo	EnSight
S/B (Top/Bottom)	1115	1093	60	61
Z-Factor	0.952	0.933	0.946	0.947
LLD [pg/mL]	4.3	5.1	308	202
EC <sub>50</sub> [pg/mL]	876	901	183308	196158
AVG high control	2442639	2415269	99788	77586
SD high control	38588	53468	1666	1298
% CV high control	1.6	2.2	1.7	1.7
AVG low control	1625	1277	1822	1332
SD low control	97	134	83	51
% CV low control	6.0	10.5	4.5	3.8

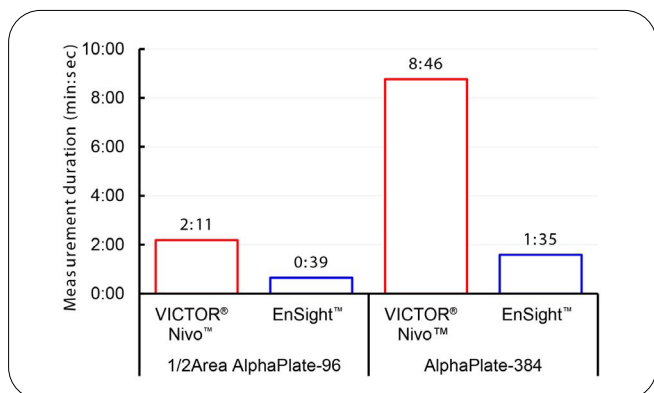


Figure 3: Comparison of Alpha measurement durations on the VICTOR Nivo and EnSight using preset protocols and different plate types (96-well, 384-well). Numbers represent the measurement duration between the first (A1) and the last well (H12 or P24). Referring to throughput, the EnSight typically exceeds the VICTOR Nivo by a factor of 3.4 (96-well) and 5.5 (384-well), respectively.

## Discussion

AlphaLISA assays for two different analytes were evaluated for critical performance metrics. Overall, the Alpha-enabled VICTOR Nivo multimode plate reader was highly competitive

in both assay quality and sensitivity parameters. In terms of measurement speed, the EnSight using an Alpha High throughput screening option is faster than the VICTOR Nivo.

The data presented here clearly show that the Alpha-enabled VICTOR Nivo multimode plate reader allows for Alpha technology using a cost-effective plate reader, with no compromises on sensitivity or data quality. As such, this reader is an ideal starter instrument when introducing Alpha technology in a lab. MyAssays Desktop analysis software comes as a standard with the VICTOR Nivo, providing preconfigured protocols for data analysis and the ability to quickly and easily build analysis modules (Pro version required). This allows users to shift their focus from instrument set up to the assay itself.

## Reference

1. Zhang, J.H. and Chung, Thomas & Oldenburg, K.R.. (1999). A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Journal of biomolecular screening. 4. 67-73. DOI 10.1177/108705719900400206