Speed up your immunoassays with AlphaLISA detection on VICTOR Nivo.

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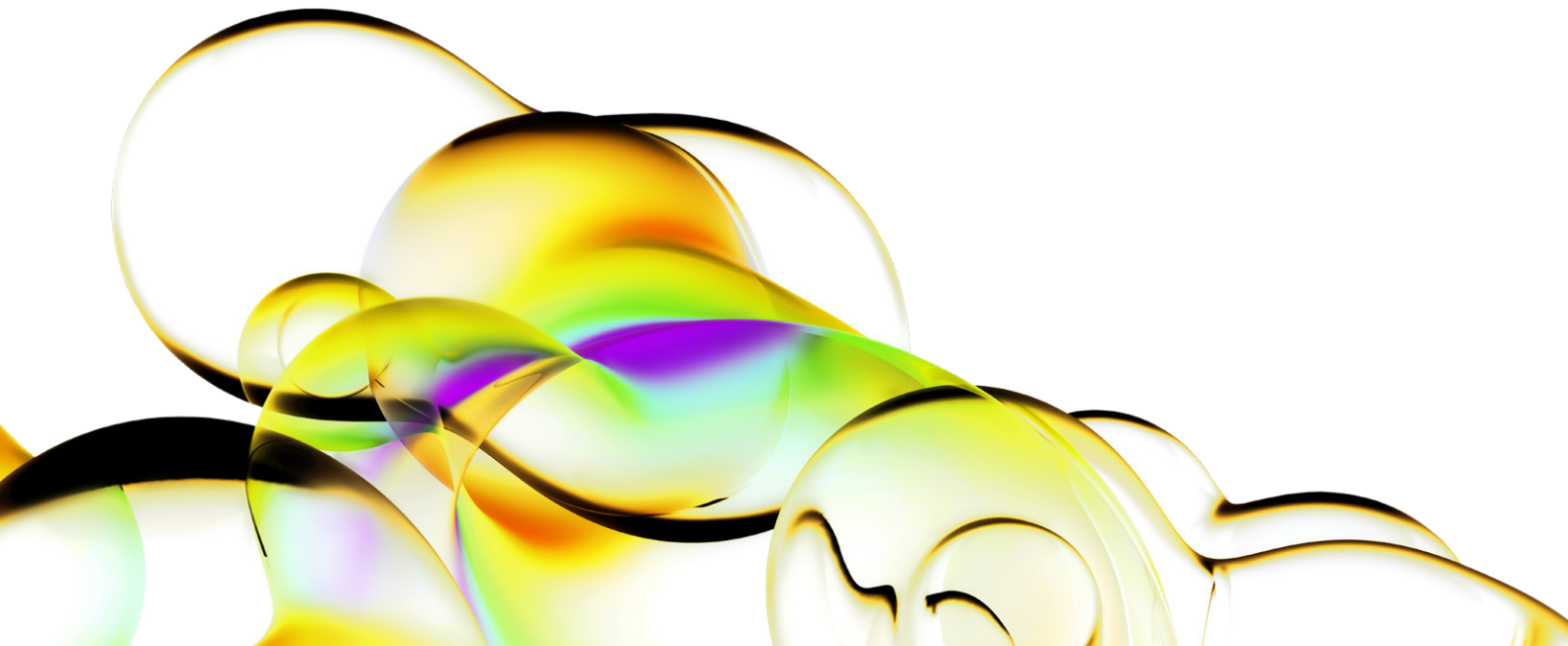
Introduction

Alpha (amplified luminescent proximity homogeneous assay) is a versatile, bead-based homogeneous assay platform for a large variety of target molecules. Alpha Technology simplifies an assay workflow and accelerates time-to-results because no wash steps are needed. Previous comparisons with ELISA (enzyme-linked immunosorbent assay) have shown that both Alpha and ELISA technologies yield comparable results[1]. While ELISA technology is considered the gold standard of immunoassays, it requires numerous wash steps which make it laborious and time-consuming. In the field of drug discovery, technologies that give robust, sensitive, automatable, and reproducible immunoassays at a lower cost are highly desirable.

To bring this technology to more research environments, the VICTOR® Nivo™ multimode plate reader has now added Alpha Technology to its array of detection capabilities with no compromises on sensitivity or data quality[2]. In order to quantify assay work-load and performance, we compared the AlphaLISA® mTNF-α assay kit to an ELISA kit based on colorimetric quantification (AlphaLISA and ELISA schematics shown in Figure 1) using the VICTOR Nivo multimode plate reader for detection.



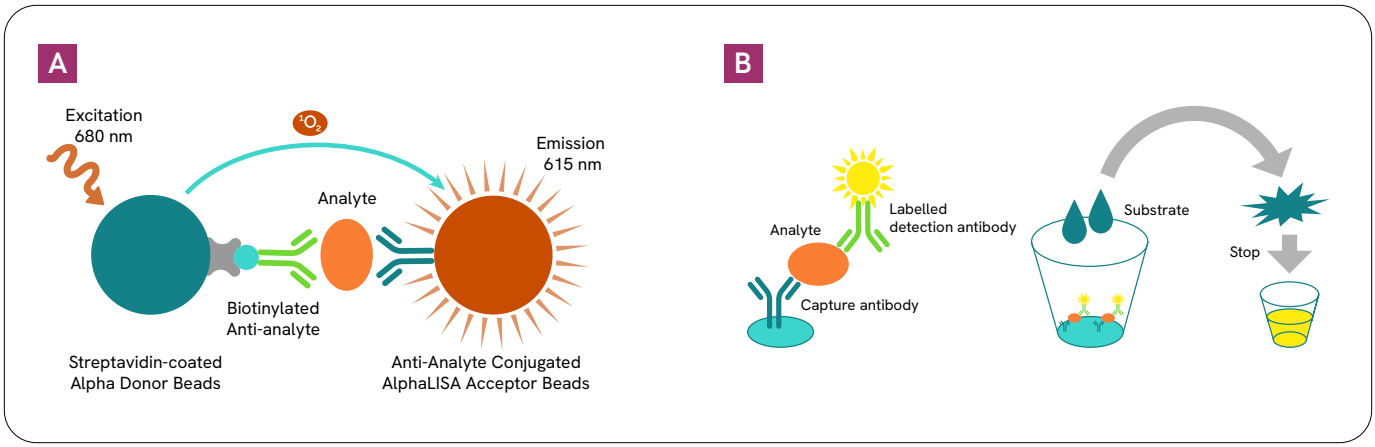


Figure 1: Comparison of AlphaLISA and ELISA assay schematics. A) In an AlphaLISA assay, one anti-analyte antibody is biotinylated and a second anti-analyte antibody is directly conjugated to AlphaLISA Acceptor beads. In the presence of the analyte of interest, Streptavidin Donor and antibody-conjugated Acceptor beads are brought into proximity, allowing the AlphaLISA signal to be generated following laser excitation at 680 nm. B) In an ELISA assay, a capture antibody specific to the antigen of interest is coupled to the microplate and another polyclonal antibody specific for mouse TNF-α is linked to an enzyme. After a series of blocking and wash steps to remove excess detection antibodies non-specifically bound, a substrate solution is added. After addition of a stop solution, Absorption is proportional to the amount of analyte in the sample.

Materials and methods

The AlphaLISA Mouse Tumor Necrosis Factor alpha (mTNF-α) Kit (#AL505 C/F), Alpha-enabled VICTOR Nivo multimode plate reader and white 384-well OptiPlates™ (#6007290) were supplied by Revvity. The colorimetric ELISA mTNF-α kit and microplates were purchased from an established ELISA kit provider. Kits were run according to each manufacturer's protocol.

Measurement parameters for the AlphaLISA and the colorimetric ELISA absorbance protocol were set according to the parameters detailed in Table 1. In both assays, a dilution series of mTNF-α was prepared in the provided assay diluents.

Results were plotted and data was fitted using a sigmoidal four-parameter logistic equation. In order to determine the lower detection limit (LDL), results from 12 blank wells were used. The LDL was calculated by interpolating the average of 12 blank wells + 3x standard deviation of 12 blank wells on the standard curve.

Results

The results of both assays are shown in Figure 2 and 3.

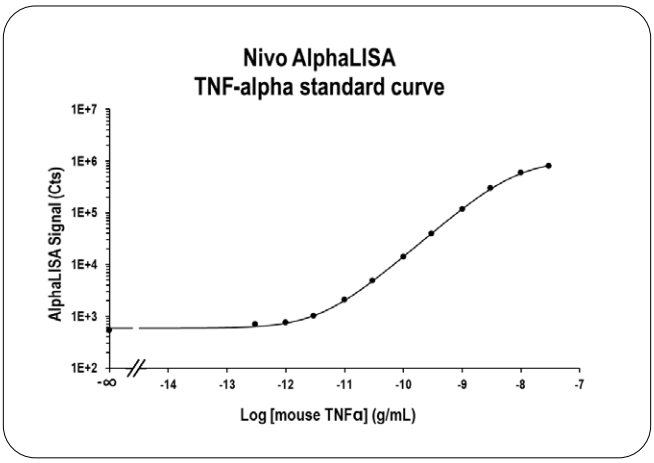


Figure 2: AlphaLISA mouse TNF-α standard curve. A lower detection limit of 1.16 pg/mL was determined in the assay.

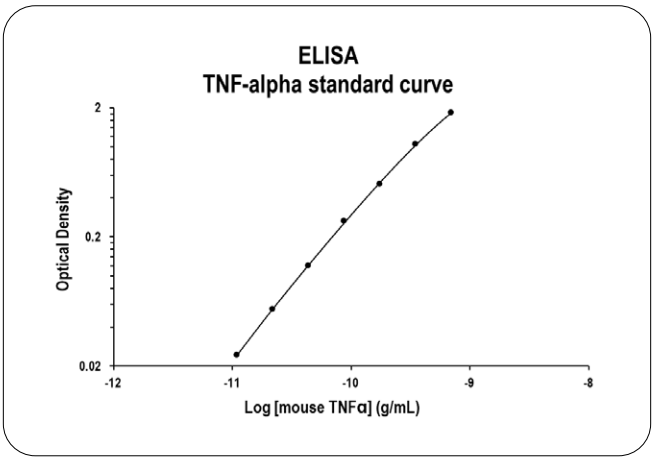


Figure 3: ELISA mouse TNF-α standard curve. A lower detection limit of 1.78 pg/mL was determined.

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

	AlphaLISA	ELISA
Detection Mode	Alpha	Absorbance
Excitation	680 nm laser @ 100% laser power	Flash lamp at 10 mJ, 450/10 nm filter
Emission	575/110 nm filter	-
Measurement Time	50 msec excitation time 100 msec emission time	500 msec
Measurement Order	Left to right by rows	Bi-directional

Both assays performed well according to their kit instructions. Key result parameters gained from the fits are depicted in Table 2 and showed almost the same lower detection limit for both assays. The EC_{50} parameter varied slightly, but still within acceptable range. However, the accessible concentration range was two orders of magnitude for ELISA vs. 3-4 orders of magnitude for AlphaLISA. With this wider range for AlphaLISA, assay development is easier because samples having wide concentration variations, e.g. primary samples, do not need to be adjusted as much to the assays detection consecration range.

Table 2: Comparison of fit result data of AlphaLISA and ELISA assay.

TNF-α assays		
	AlphaLISA	ELISA
LDL (pg/mL)	1,16	1,78
EC_{50} (pg/mL)	8215	1383
S/B	1498	75
Assay Detection Range	3-4 orders of magnitude	two orders of magnitude

Comparison of Assay Protocols

AlphaLISA is a homogeneous assay format without any wash steps. This keeps the protocol simple and requires fewer pipetting steps when compared to the ELISA. A detailed comparison of the AlphaLISA and ELISA mouse TNF-α assay protocols is shown in Table 3. The AlphaLISA mouse TNF-α assay can be run in approximately 2.5-3 hours, whereas the ELISA mouse TNF-α assay had a total assay time of 5-6 hours and requires eight washes.

Conclusion

With the addition of Alpha Technology, the VICTOR Nivo multimode plate reader has all major detection technologies to run your everyday biochemical and cell-based assays. Using the mTNF-α quantification example, we showed that traditional ELISA and AlphaLISA assays deliver comparable results regarding assay sensitivity. The AlphaLISA technology brings the additional advantage of a quicker and less laborious assay workflow, due to the no-wash assay platform. The overall time-to-results is reduced by approximately 50% for the AlphaLISA approach with slightly improved sensitivity. Without the need of several wash steps, the assay robustness and reproducibility is also improved. In addition, MyAssays Desktop software makes it easy to analyze the data with only a few clicks using the readily available analysis protocols. There is no need to spend extra time trying to figure out your own analysis steps.

Table 3: Comparison of AlphaLISA and ELISA protocols.

Step	AlphaLISA	ELISA
1	Add 5 µL of each analyte standard dilution or 5 µL of sample	Add 50 µL of Assay Diluent
2	Add 10 µL of 5X Anti-Analyte Acceptor beads	Add 50 µL of standard, control, or sample. Mix by gently tapping the plate frame for 1 minute.
3	Incubate 30 minutes at 23 ˚C	Incubate for 2 hours at room temperature
4	Add 10 µL of 5X Biotinylated Antibody Anti-analyte	Wash plate x4
5	Incubate 60 minutes at 23 ˚C	Add 100 µL of Mouse TNF-α Conjugate
6	Add 25 µL of 2X SA-Donor beads	Incubate for 2 hours at room temperature
7	Incubate 30 minutes at 23 ˚C in the dark	Wash plate x4
8	Read using VICTOR Nivo plate reader with Alpha	Add 100 µL of Substrate Solution
9		Incubate for 30 minutes at room temperature. Protect from light.
10		Add 100 µL of Stop Solution
11		Read on VICTOR Nivo plate reader at 450 nm and 570 nm within 30 minutes
Overall	2.5-3 hours overall, no wash steps	5-6 hours overall, 8 washes

The VICTOR Nivo can be integrated easily in any lab to save space and reduce cost. It packs all major detection technologies in the industry's smallest benchtop footprint and still offers excellent sensitivity and versatility as expected from a multimode plate reader.

References

1. Bagna Bao and Catherine Lautenschlager (2018). Detection of Human PD-L2 in Serum - Comparison of AlphaLISA and ELISA Immunoassays. Revvity. Technical Note.

2. Urban Maier and Norbert Garbow (2019). High-Performance Alpha Technology on VICTOR Nivo. Revvity. Technical Note.

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