

Quantification of human TNF- α using VICTOR Kira monochromator-based multimode plate reader.

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VICTOR Kira monochromator-based multimode plate reader



Introduction

Tumor necrosis factor-alpha (TNF- α) is a key pro-inflammatory cytokine involved in immune regulation, inflammation, and disease pathogenesis. Accurate quantification of TNF- α is essential for immunology research, biomarker studies, and therapeutic development. ELISA remains the gold standard for cytokine measurement due to its sensitivity and reproducibility. This application note demonstrates the performance of the VICTOR Kira™ multimode plate reader for human TNF- α ELISA using the LEGEND MAX™ Human TNF- α ELISA Kit (cat. no.: 430207).

The VICTOR Kira reader offers several advanced features that make it ideal for ELISA and other biochemical assays. Its monochromator-based optics, covering 230–1000 nm, provide flexibility for precise wavelength selection. The instrument supports an optical density (OD) range of up to 4.0, ensuring accurate readings even at saturation. Low detection noise enhances sensitivity, while endpoint and kinetic reading modes allow versatility of the assay. Additional features, such as plate shaking and temperature control, contribute to reproducibility and consistent assay performance.

Materials and methods

The LEGEND MAX Human TNF- α ELISA Kit (cat. no.: 430207) includes a pre-coated 96-well strip microplate, detection antibody, human TNF- α standard, Avidin-HRP solution, assay buffer, wash buffer (20X), substrate solution, stop solution, and plate sealers. The standard range for the assay is 15.6–1000 pg/mL. The procedure involves adding standards and samples to pre-coated wells, incubating with detection antibody and Avidin-HRP, followed by substrate addition and stop solution. Absorbance was measured at 450 nm with a correction wavelength of 570 nm using the VICTOR Kira multimode plate reader.



Figure 1: LEGEND MAX kit, BioLegend

Data analysis

Data analysis was performed using GraphPad Prism 10, which enabled nonlinear regression for curve fitting, calculation of R^2 values, and interpolation of unknown sample concentrations.

The 4PL curve fitting graph can also be plotted with the VICTOR Kira control software.

Results

The standard curve for human TNF-α showed excellent performance with a correlation coefficient (R^2) of 0.99, confirming assay reliability. One unknown sample was analyzed, yielding an OD of 0.079, corresponding to a calculated concentration of 113.4 pg/mL. These results demonstrate accurate quantification within the assay’s dynamic range.

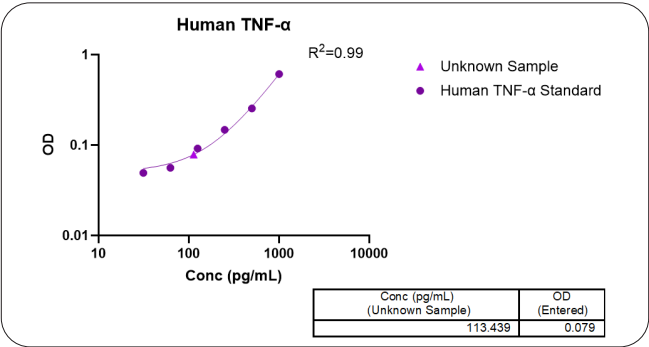


Figure 2: Human TNF-α ELISA standard curve

Conclusion

The VICTOR Kira reader delivered accurate detection across the full concentration range and demonstrated excellent reproducibility, aided by plate shaking and temperature control. Its monochromator optics allow dual-wavelength correction, which is essential for minimizing background interference. This study confirms the suitability of the VICTOR Kira multimode plate reader for ELISA assays. The measured dynamic range and assay linearity were in line with the LEGEND MAX Human TNF-α ELISA Kit manual.

