APPLICATION BRIEF

Quantification of human IFN-γ using VICTOR Kira monochromator-based multimode plate reader.

Authors

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Introduction

Interferon-gamma (IFN-γ) is a critical cytokine involved in innate and adaptive immunity, playing a key role in activating macrophages and regulating immune responses against pathogens and tumors. Accurate quantification of IFN-γ is essential for immunology research, vaccine development, and therapeutic monitoring. Enzyme-linked immunosorbent assay (ELISA) remains the gold standard for cytokine measurement due to its high sensitivity, specificity, and reproducibility. This application note demonstrates the performance of the VICTOR Kira™ multimode plate reader for human IFN-γ ELISA using the LEGEND MAX™ Human IFN-γ ELISA Kit (cat. no.: 430107)

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 for assay versatility. Additional features, such as plate shaking and temperature control, contribute to reproducibility and consistent assay performance.

VICTOR Kira monochromator-based multimode plate reader

Materials and methods

The LEGEND MAX Human IFN-γ ELISA Kit (cat. no.: 430107) includes a pre-coated 96-well strip microplate, detection antibody (12 mL), human IFN-γ standard (20.1 ng), Avidin-HRP solution (12 mL), 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 the pre-coated wells, incubating with detection antibody and Avidin-HRP, followed by substrate addition and the 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.

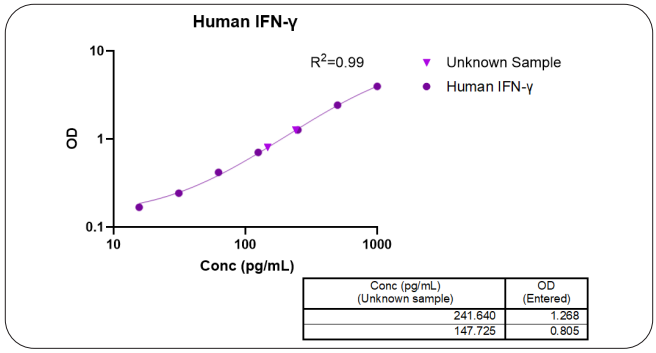
The 4PL curve fitting graph can also be plotted with VICTOR Kira control software.

Results

The human IFN-γ standard curve was generated using a four-parameter logistic (4PL) model on a log-log scale, yielding an excellent fit (R^2 = 0.99) and indicating strong agreement between observed and predicted values.

Two unknown samples were analyzed. The first sample produced an OD of 1.268, corresponding to an interpolated concentration of 241.64 pg/mL, while the second sample yielded an OD of 0.805, corresponding to 147.73 pg/mL. Both values fall within the central portion of the standard curve, confirming their suitability for accurate interpolation.

These results indicate that the LEGEND MAX ELISA Kit provides accurate and reproducible quantification of IFN-γ when used with the VICTOR Kira reader.



Conc (pg/mL) (Unknown sample)	OD (Entered)
241.640	1.268
147.725	0.805

Figure 2: Human IFN-γ 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 IFN-γ ELISA Kit manual.