APPLICATION BRIEF

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

Authors

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Introduction

Enzyme-linked immunosorbent assays (ELISAs) are widely used for quantifying cytokines in biological samples. The LEGEND MAX™ Mouse IFN-γ ELISA Kit (cat. no.: 430807) is designed for sensitive and specific detection of mouse interferon gamma (IFN-γ). This application note demonstrates the performance of the VICTOR Kira™ multimode plate reader for mouse IFN-γ ELISA using the LEGEND MAX Mouse IFN-γ ELISA Kit.

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 Mouse IFN-γ ELISA Kit (cat. no.: 430807) includes a pre-coated 96-well strip microplate, detection antibody, mouse IF-γ standard, Avidin-HRP solution, assay buffer, wash buffer (20X), substrate solution, stop solution, and plate sealers. The standard range for the assay is 31.2-2000 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.

VICTOR Kira monochromator-based multimode plate reader

Figure 1: LEGEND MAX kit, BioLegend

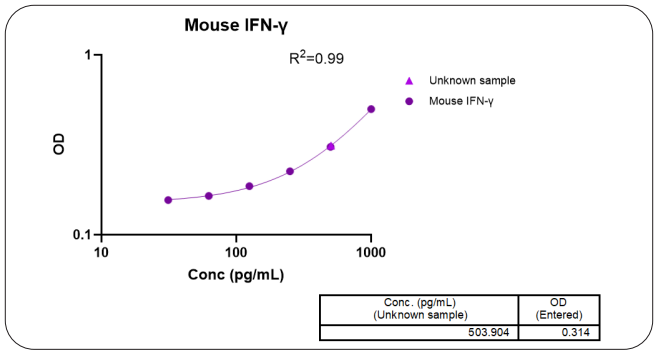

Figure 2: Mouse-IFN-γ ELISA standard curve

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 exhibited a sigmoidal shape with excellent correlation (R^2 = 0.99), confirming the robustness of the assay. The dynamic range spanned approximately two orders of magnitude, ensuring accurate quantification across physiological concentrations. The VICTOR Kira reader provided stable readings with minimal background noise and demonstrated compatibility with the kit's recommended wavelength settings.

An unknown sample produced an OD of 0.314, corresponding to an interpolated concentration of 503.90 pg/mL, which falls within the mid-upper range of the 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 Mouse IFN-γ ELISA Kit manual.