Quantification of human granzyme B using VICTOR Kira monochromator-based multimode plate reader.

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Introduction

Human granzyme B is a serine protease involved in immune-mediated apoptosis and serves as a key biomarker in immunology research. Accurate quantification is essential for studies on cell-mediated cytotoxicity, inflammation, and therapeutic monitoring. This application note demonstrates the performance of the VICTOR Kira™ multimode plate reader for human granzyme B ELISA using BioLegend from Revvity's LEGEND MAX™ Human Granzyme B ELISA Kit. (cat. no.: 439207)

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

Materials and methods

The LEGEND MAX Human Granzyme B ELISA Kit (cat. no.: 439207) includes a pre-coated 96-well strip microplate, detection antibody (12 mL), human granzyme B standard (20.2 ng), Avidin-HRP solution (12 mL), assay buffer, wash buffer (20X), substrate solution, stop solution, and plate sealers (Figure 1).

The standard range for the assay is 9.4–600 pg/mL. The procedure involves adding standards and samples to pre-coated wells, incubating with a detection antibody and Avidin-HRP, then adding the substrate and stop solution. Absorbance is measured at 450 nm with a correction wavelength of 570 nm using the VICTOR Kira reader.

VICTOR Kira monochromator-based multimode plate reader

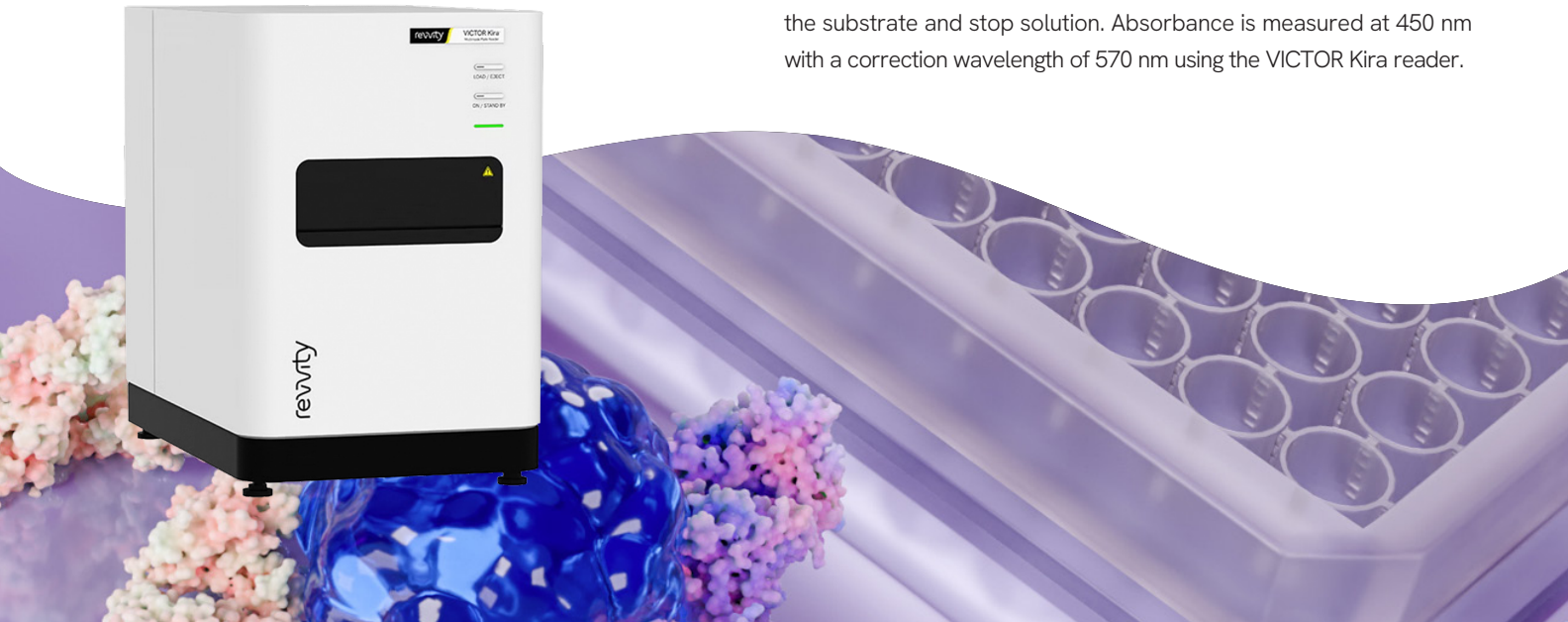

Figure 1: LEGEND MAX kit, BioLegend

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 human granzyme B 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.

The unknown sample was assayed in duplicate, yielding identical optical density (OD) values of 0.120 at 450 nm.

Interpolation from the 4PL curve indicated a concentration of 29.14 pg/mL. The curve exhibited the expected sigmoidal shape with a well-defined linear segment in the mid-range, ensuring accurate interpolation of unknowns.

The dynamic range of the assay spanned from the lowest standard 9.4 pg/mL to the highest standard approaching 600 pg/mL, providing robust coverage for both low and high concentrations. This broad dynamic range, combined with high linearity in the central portion of the curve, confirms the assay's suitability for precise quantification in VICTOR Kira across physiologically relevant concentrations.

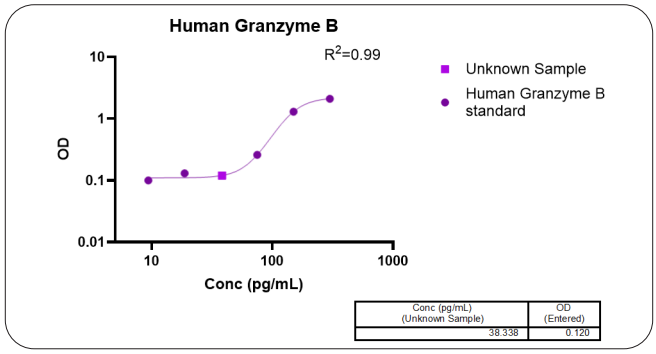

Figure 2: Human granzyme B ELISA standard curve

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 Granzyme B ELISA Kit manual.