APPLICATION NOTE

PLPro enzyme inhibition assay using VICTOR Kira multimode plate reader.

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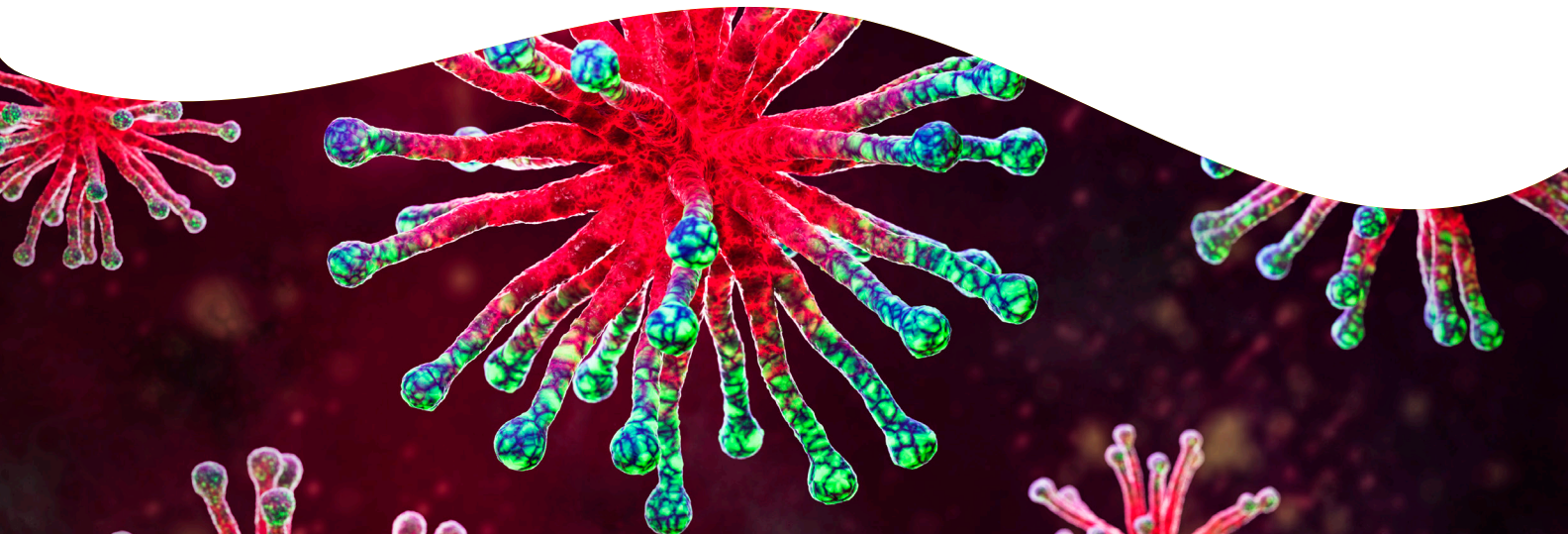
Introduction

PLPro (Papain-Like Protease) is a critical target for antiviral drug development due to its role in viral replication and modulation of host immune responses. Inhibiting PLPro can suppress viral replication and reduce dysregulation of signaling cascades in infected cells. This application note demonstrates the performance of the VICTOR Kira™ monochromator-based multimode plate reader for PLPro inhibition assays compared to other multimode readers and filter-based systems. The PLPro substrate is a small 5-mer fluorogenic peptide that releases the C-terminal AMC dye (7-amido-4-methylcoumarin) upon proteolysis. The liberated AMC generates fluorescence, which is monitored at an excitation wavelength of 345 nm and an emission wavelength of 445 nm. The degree of fluorescence correlates with PLPro activity, and inhibition by test compounds reduces the signal.

Materials and methods

The assay was performed using a fluorogenic PLPro substrate in a 384-well plate format. Compound X was tested across a range of concentrations to generate a dose-response curve. Fluorescence intensity was measured using the VICTOR Kira multimode plate reader, which features a quad monochromator system with an excitation range of 230–825 nm and an emission range of 245–850 nm, making it highly suitable for this assay. This configuration allows precise wavelength selection and minimizes background interference. For comparison, the same assay was run on other multimode readers and a filter-based system under identical conditions. All measurements were performed in endpoint mode, and plates were incubated at room temperature with appropriate shaking to ensure uniform reaction conditions.

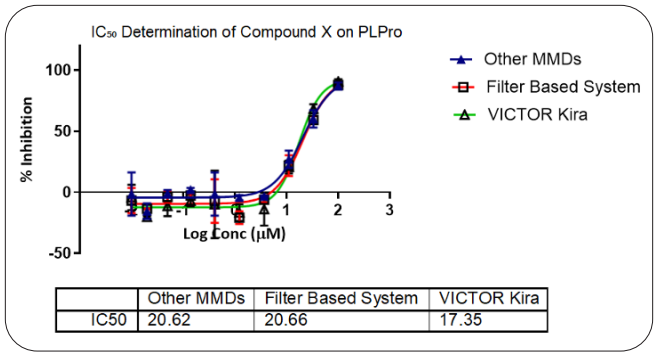


Data analysis

Data analysis was performed using GraphPad Prism 10. RFU values were converted into % inhibition relative to positive and negative controls. Dose-response curves were fitted using nonlinear regression to calculate IC_{50} values. VICTOR Kira achieved an IC_{50} of 17.35 µM, compared to 20.6 µM for other multimode readers and 20.7 µM for the filter-based system. The signal-to-background (S/B) ratio was highest for VICTOR Kira (22.1), indicating superior sensitivity, and the Max/Min ratio was also highest (7.0), confirming better dynamic range.

Results

Figure 1 shows the dose-response curve for Compound X against PLPro, expressed as % inhibition versus log concentration (µM). VICTOR Kira demonstrated an IC_{50} of 17.35 µM, compared to 20.6 µM for other multimode readers (MMDs) and 20.7 µM for the filter-based system. The signal-to-background (S/B) ratio was highest for VICTOR Kira (22.1), indicating superior sensitivity, and the Max/Min ratio was also highest (7.0), confirming better dynamic range. These results highlight the improved performance of VICTOR Kira in detecting fluorescence signals with greater accuracy and sensitivity.



	Other MMDs	Filter Based System	VICTOR Kira
IC50	20.62	20.66	17.35

Figure 1: Dose-response curve for PLPro inhibition across different plate readers.

	Other MMDs	Filter based system	VICTOR Kira
IC_{50}	20.6	20.7	17.4
Max/Min	4.6	5.2	7.0
S/B	11.1	11.1	22.1

Figure 2: Performance metrics for PLPro enzyme inhibition on different detection systems.

Conclusion

VICTOR Kira delivers robust and sensitive performance for PLPro inhibition assays, offering advantages in IC_{50} accuracy, signal-to-background ratio, and dynamic range. Its advanced capabilities make it suitable for high-throughput screening and antiviral drug development workflows.

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