APPLICATION NOTE

Comparative evaluation of kinase inhibition using ADP-Glo™ luminescent assay on filter-based system and VICTOR Kira.

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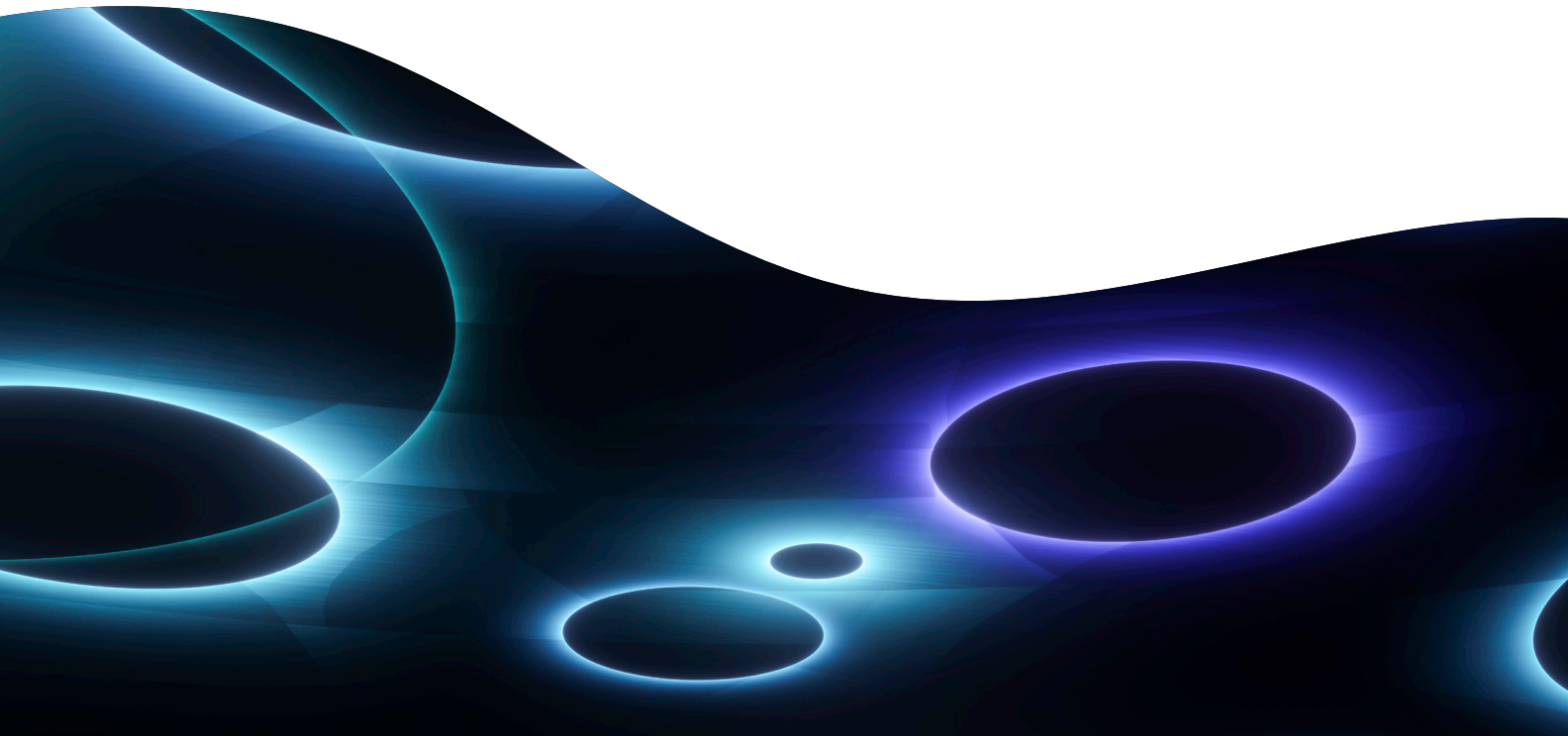
Kinase inhibition is a critical parameter in drug discovery, and accurate determination of IC_{50} values is essential for characterizing inhibitor potency. This study employed the ADP-Glo™ Kinase Assay to estimate the IC_{50} of inhibitor compound X against a target kinase using two multimode plate readers: a filter-based system and the VICTOR Kira™. Luminescence generated from ADP conversion to ATP was measured on both instruments, and dose-response curves were analyzed to calculate IC_{50} values. The results demonstrate comparable performance between the two instruments, confirming assay robustness and reproducibility.

Protein kinases regulate key cellular processes, and their dysregulation is implicated in numerous diseases, including cancer. Small-molecule kinase inhibitors are widely studied as therapeutic agents, and determining their IC_{50} values is a fundamental step in drug development. Traditional kinase assays often involve radioactive or colorimetric methods, which can be labor-intensive and hazardous. The ADP-Glo™ Kinase Assay provides a sensitive, luminescent alternative for quantifying ADP produced during kinase reactions (Figure 1). This study aimed to estimate the IC_{50} of an unknown compound using the ADP-Glo™ assay and compare performance across two instruments—an in-house filter-based system and the VICTOR Kira, a monochromator-based system.



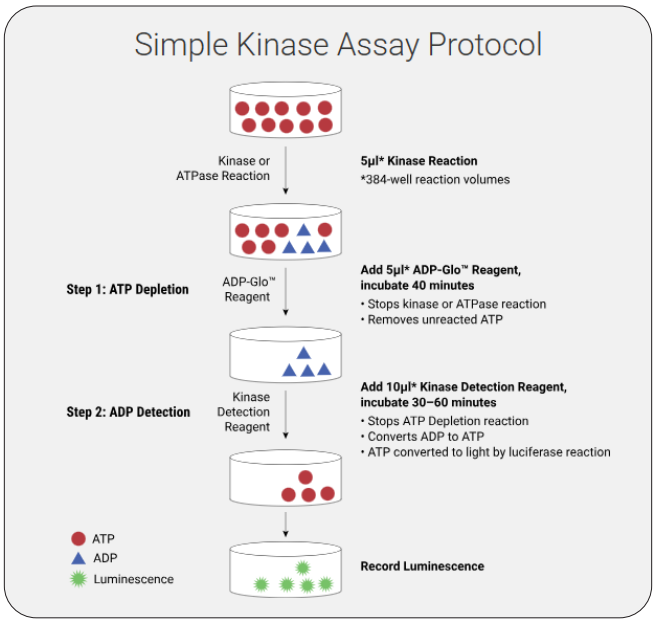


Figure 1: Standard Kinase workflow

Materials and methods

The ADP-Glo™ Kinase Assay (Promega, V9103) was used to measure kinase activity and inhibition. The assay principle involves converting ADP generated during the kinase reaction into ATP, which drives a luciferase reaction producing luminescence proportional to kinase activity. Kinase reactions were performed in 5 µL volumes under optimized conditions. After completion, ADP-Glo™ reagent was added to terminate the reaction and deplete remaining ATP, followed by the addition of the kinase detection reagent to convert ADP into ATP and initiate the luciferase reaction. Luminescence was recorded using two instruments, a filter-based system and the VICTOR Kira in a microplate format.

Data analysis

Dose-response curves were generated by plotting percent inhibition against the logarithmic concentration of the compound. IC_{50} values were calculated using nonlinear regression in GraphPad Prism 10, and assay performance was assessed by determining signal-to-background ratios and dynamic range (Max/Min luminescence).

Results

The ADP-Glo™ Kinase Assay produced clear luminescent signals correlating with kinase inhibition across both instruments. Dose-response curves for the compound exhibited sigmoidal profiles, confirming effective inhibition of the target kinase. IC_{50} values were nearly identical between instruments: **1.239 µM on the filter-based system and 1.234 µM on the VICTOR Kira**, demonstrating equivalent inhibitor potency across platforms (Figure 2). The dynamic range was slightly higher on the VICTOR Kira than on the filter-based reader, while signal-to-background ratios were robust for both instruments (Figure 3), reflecting excellent assay sensitivity and reproducibility. These findings confirm that the VICTOR Kira performs on par with, and in some aspects surpasses, traditional filter-based readers for IC_{50} determination in luminescent kinase assays.

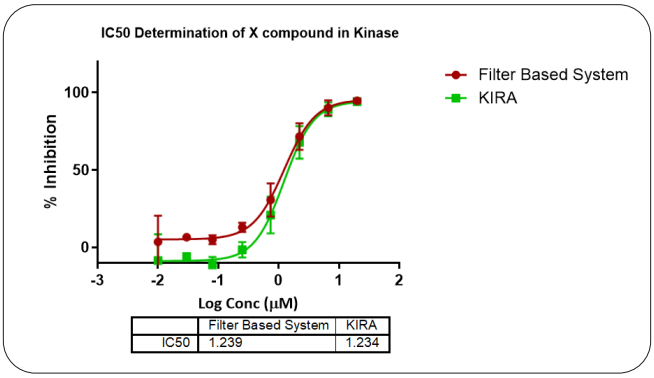


Figure 2: IC_{50}: Filter Based System vs VICTOR Kira

	FBS	KIRA
IC50	1.24	1.23
Max/Min	14.7	16.9
S/B	34.1	34.7

Figure 3: IC_{50}: Filter Based System (FBS) vs VICTOR Kira