APPLICATION NOTE

# Rapid quantification of lactate dehydrogenase (LDH) activity using VICTOR Kira multimode plate reader.


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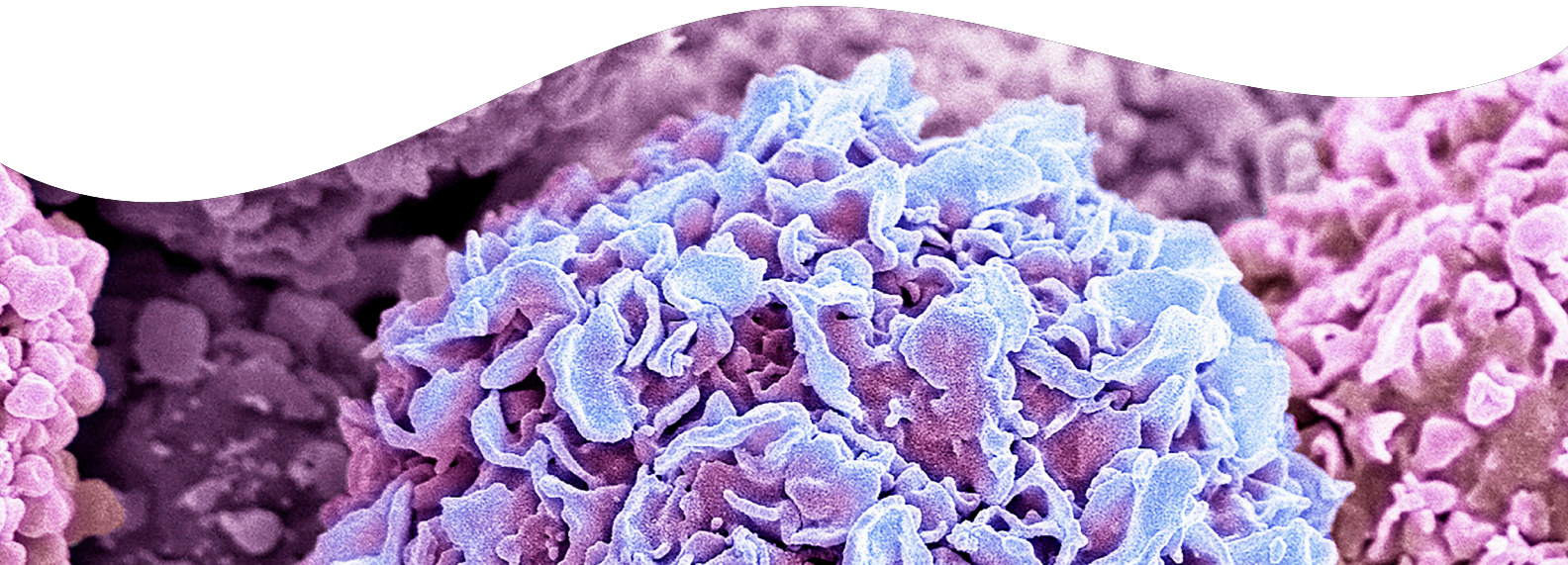
## Introduction

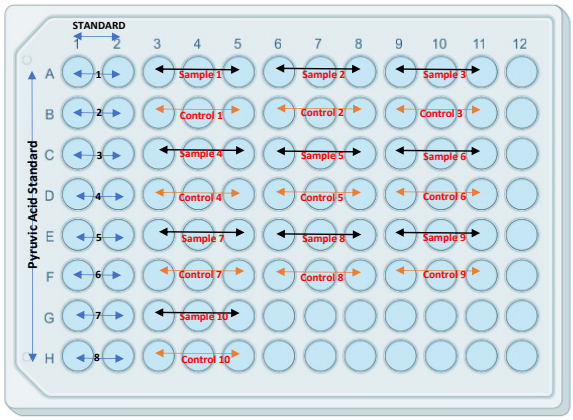
The lactate dehydrogenase (LDH) activity assay is a standard method in cell-based assays for screening for the cytotoxic effects of compounds. LDH is a cytoplasmic enzyme found in almost all living cells. It catalyzes the cyclic conversion of lactate to pyruvate in the presence of coenzyme I. In a cytotoxicity study, the LDH activity assay stands in as the most reliable and fast method to investigate whether the cytotoxicity of the tested compound is correlated with cell membrane damage.

Here, we investigated the lactate dehydrogenase activity in MCF-7 breast cancer following treatment with test compounds (IxF and IxL) by measuring the LDH activity on the VICTOR Kira™ multimode plate reader.

VICTOR Kira is a monochromator-based multimode plate reader with advanced technology for measuring absorbance, fluorescence, and luminescence across diverse samples. The instrument offers versatile functionality, simple wavelength selection, and built-in data analysis features. The system is simplified, which allows you to select and modify protocols according to individual needs. It is an ideal instrument for labs with multiple users.

In the present study, LDH released into the cell culture medium was quantified by measuring the absorbance value at 450 nm on the VICTOR Kira instrument.






| | STANDARD | SAMPLE | CONTROL |
|---|---|---|---|
| Pyruvic Acid Standard (µL) | 20 | - | - |
| Sample (µL) | - | 20 | 20 |
| Double distilled water (µL) | 5 | - | 5 |
| Coenzyme I (µL) | - | 5 | - |
| Substrate buffer (µL) | 25 | 25 | 25 |
| | Mix and incubate for 15mins at 37°C | | |
| Chromogenic (µL) | 25 | 25 | 25 |
| | Mix and incubate for 15mins at 37°C | | |
| Alkali Solution (µL) | 200 | 200 | 200 |


**Absorbance readout at 450nm**

Figure 1: Representing 96-well plate mapping and reaction setup. Standard wells consist of Pyruvic acid. Sample and Control wells have IxF treated cell culture supernatant. The reaction mixture constitute of substrate buffer, coenzyme I, chromogenic reagent and alkali solution.

## Materials and methods

MCF-7 breast cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1.0 g/L glucose, sodium bicarbonate, and sodium pyruvate and supplemented with 12% Fetal Bovine Serum (FBS), 2mM L-glutamine, and antibiotic solution (10,000 U Penicillin and Streptomycin). For the LDH assay, MCF-7 cells were washed with 1X PBS (pH 7.4), trypsinized with 1X trypsin EDTA solution, and resuspended in fresh DMEM medium to prepare a cell suspension of 1.4 x $10^4$ cells/mL. 100 µL of cells were seeded in a 96-well microtiter plate and incubated at 37°C for 24 h in a $CO_2$ incubator according to the standard protocol. After 24 h of incubation, the test compounds (IxF and IxL) were added at different concentrations ranging from 5 to 320 µg/mL in triplicate and further incubated for an additional 24 h at 37°C in a $CO_2$ incubator. Doxorubicin (Sigma Aldrich) at 10 µg/mL concentration was used as a positive control. Next, the supernatant was collected to perform the LDH assay.

## Lactate dehydrogenase assay (LDH)

To investigate whether IxF and IxL show cytotoxic activity by compromising cell membrane integrity of breast cancer cells, the LDH enzyme released into the medium after treatment was measured using the Lactate Dehydrogenase (LDH) Activity Assay Kit (Catalog Number: EEA013). The assay was performed as described in the kit manual and as represented in Figure 1. A serial dilution of Pyruvic Acid Standard (2 µmol/mL) was prepared in a range of 0.0-1.0 µmol/mL in autoclaved distilled water. Add 20 µL of each standard and 5 µL of double distilled water into a microplate (Invitrogen, 96-well microplate) in duplicates. In sample and control wells, add 20 µL of sample (supernatant collected after treating MCF-7 cells with different concentrations of IxF and IxL and 10 µg/mL of doxorubicin). Next, add 25 µL of substrate buffer to each well and 5 µL of coenzyme I to the sample well. Instead of coenzyme I, I add 5 µL of double distilled water in control wells. Mix carefully and incubate at 37°C for 15 min. Further, add 25 µL of chromogenic agent to all wells. Again, mix well and incubate at 37°C for 15 min. Finally, add 200 µL of alkali reagent solution to each well and let it stand at room temperature for 5 min. Measure the absorbance value at 450 nm on VICTOR Kira and a filter-based multimode plate reader instrument. LDH activity (U/L) for each concentration was calculated using a standard curve graph of pyruvic acid.

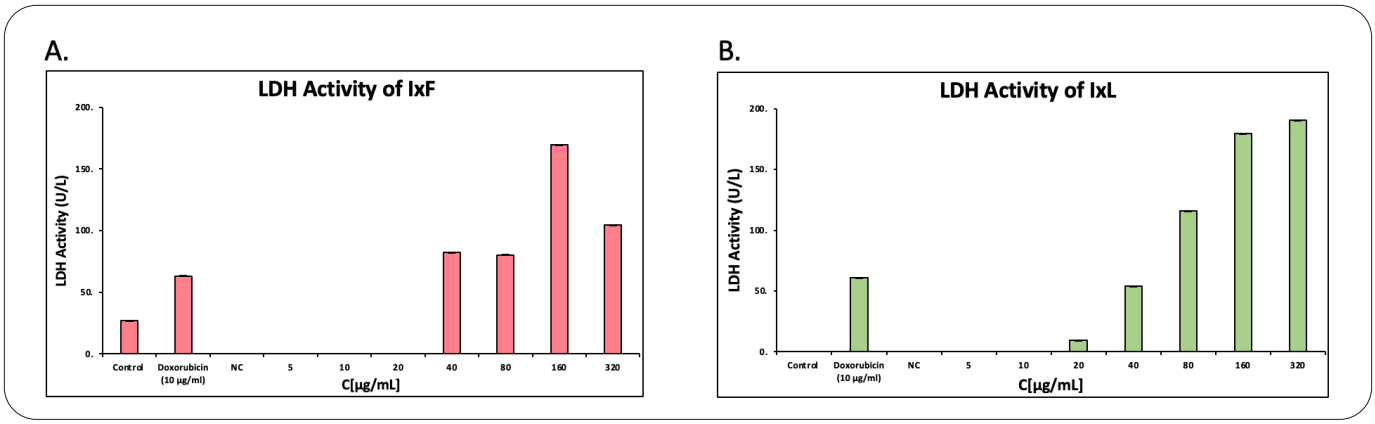



Figure 2: Effect of LDH Activity (U/L) of test compound against MCF-7 cells. Lactate dehydrogenase activity (U/L) of A. IxF and B. IxL tested at different concentration (50-320 µg/mL) against MCF-7 cells. Doxorubicin (10 µg/mL) was used as positive control and untreated cells served as the control. Distilled water was used as negative control. Increased LDH Activity (U/L) was observed in dose-dependent manner of test compound.

## Results and discussion

In order to investigate compound-related cytotoxic effects, a lactate dehydrogenase activity (LDH) assay was performed. Absorbance reading was measured on the monochromator-based VICTOR Kira multimode plate reader and a filter-based multimode system to compare and check the suitability and reliability of both systems.

## Lactate dehydrogenase (LDH) activity assay

The Lactate dehydrogenase activity assay measures the amount of pyruvate formed by the activity of the LDH enzyme released from the cell membrane of non-viable cells into the medium. Once released into the medium, the LDH enzyme catalyzes the cyclic conversion of lactate (substrate) to pyruvic acid in the presence of coenzyme I. Pyruvate formed reacts with the chromogenic agent to give a red-brown color in an alkaline solution. The color intensity is directly proportional to the pyruvate amount, which indirectly measures the LDH enzyme activity. Ultimately, LDH acts as a marker for cell membrane damage, the most reliable and fast method to evaluate the cytotoxic effect of a test compound on cancer cells. In this study, we measure the release of LDH from MCF-7 breast cancer cells treated with the test compound at different concentration ranges (5–320 µg/mL). The LDH activity assay shows prominent dose-dependent LDH activity in cells treated with IxF and IxL at 24 h of exposure (Figure 2). The LDH activity as measured on VICTOR Kira at the selected 450 nm absorbance wavelength, was found to be in the range of 50-200 U/L for the given test samples. Doxorubicin, an effective chemotherapeutic drug, showed 54 U/L of LDH activity at 10 µg/mL. The assay demonstrated a broad dynamic range of detection by measuring test compound-mediated cytotoxic activity at different concentrations, confirming the robustness and sensitivity of the assay using VICTOR Kira. We investigated and found IxF and IxL compromise cell membrane integrity of MCF-7 breast cancer cells. This finding further demonstrates that the cytotoxic effect of the test compound is via cell membrane disruption.

## Comparative study of the pyruvic acid standard curve using VICTOR Kira and a filter-based plate reader

To estimate instrument performance, sensitivity, and reliability, a pyruvic acid standard curve was generated using both the monochromator-based VICTOR Kira and the filter-based multimode plate reader. The microtiter plate was read at exactly 450 nm using VICTOR Kira, while in filter-based mode, a 450/10 nm filter was applied to determine the absorbance (OD value). The result showed no significant difference between the OD values recorded by using the two instruments. The pyruvic acid standard curve graph obtained from both systems demonstrated comparable absorbance intensities and an equivalent coefficient of determination ($R^2$ = 0.973) (Figure 3).

This confirms both instruments provided similar results for the pyruvic acid standard, indicating that the assay can be performed on either of the instruments. But this may vary depending upon the sample that needs to be analyzed.

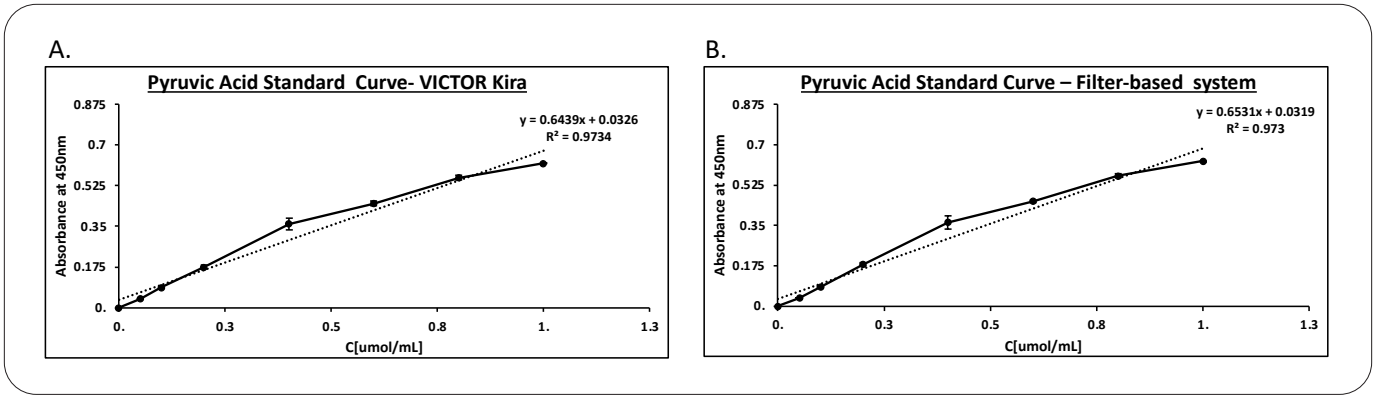



Figure 3: Comparative analysis of pyruvic acid standard curve using two instruments. No difference was observed in OD values taken on (A) VICTOR Kira and (B) filter-based multimode plate reader system. $R^2$ was found to be 0.973, as measured on both the systems.

Even though both instruments provided consistent results, VICTOR Kira offers technical advantages over filter-based systems by employing higher accuracy in wavelength selection, being capable of scanning full spectra, and having easy data acquisition, making it a superior platform for undertaking research.

## Conclusion

In this study we demonstrated the application of the VICTOR Kira multimode plate reader to efficiently quantify lactate dehydrogenase (LDH) activity. VICTOR Kira instrument provided sensitive, robust, and reliable results with minimum background interference and high-stability detection signals. The instrument's exact wavelength selection and compatibility with 96-well microtiter plates enabled multiple sample processing, making it suitable for high-throughput screening of compounds.

Overall, the method proved to be a rapid and efficient approach for evaluating LDH activity of cytotoxic compounds using the VICTOR Kira multimode detection system.

## References

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