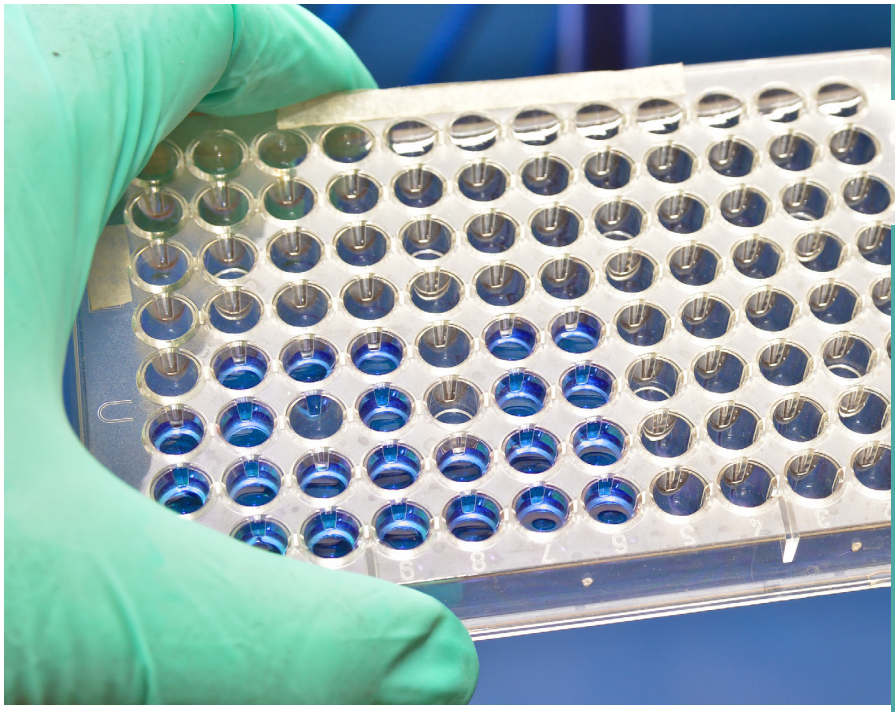

APPLICATION NOTE

Multimode Detection

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Rapid and Easy Quantification of GAPDH using the VICTOR Nivo Multimode Plate Reader and the MaxDiscovery GAPDH ELISA Kit

Introduction

The VICTOR Nivo™ multimode plate reader is a compact benchtop system, providing up to five detection modes: absorbance, luminescence, fluorescence intensity, time-resolved fluorescence and fluorescence polarization. The system is designed for busy multi-user environments and is characterized by its small footprint, its flexibility and its pre-loaded, user-friendly protocols.

In this application note, we describe the use of the VICTOR Nivo multimode plate reader and the MaxDiscovery™ Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) ELISA Kit to detect GAPDH, one of the key enzymes of glycolysis.

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used methodology for detection of, for example, proteins, peptides or antibodies in a mixture of other substances and components. It provides sensitive and accurate results in diagnostics as well as in research. ELISA is based on the principle of an antigen or antibody bound to a surface interacting specifically with the component of interest in a sample. Components which are not recognized by the bound antigen or antibody are washed away. The specific interacting pair is visualized by the selected detection system e.g. fluorescence, absorbance, radiometric, luminescence.

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The assay detection system in the MaxDiscovery GAPDH ELISA kit is based on oxidation of the 3,3',5,5'-Tetramethylbenzidine (TMB) substrate by hydrogen peroxide catalyzed by the Horseradish Peroxidase (HRP) conjugated antibody. Oxidized TMB absorbs at 370 and 652 nm. The blue color changes to yellow after addition of an acid, and the absorbance at 450 nm, detected using the VICTOR Nivo system, is proportional to the amount of GAPDH in the sample. In this study, using the GAPDH standard provided in the kit, the linear range of the assay was tested.

Material and Methods

The GAPDH ELISA was performed according to the manufacturer's protocol (MaxDiscovery GAPDH ELISA Kit, #3401, Bioo Scientific).[1] Reagents were allowed to equilibrate to room temperature, except the GAPDH standard and HRP-conjugated antibody. An 8-point dilution series of the GAPDH standard was prepared in triplicate in Secondary Dilution Reagent starting with 600 ng/mL. 100 µL were transferred to each well of a 96-well plate and incubated for 1 h at 37 °C, followed by five washes, each with 250 µl/well of 1x Wash Solution, and then addition of 100 µL/well of GAPDH Antibody-HRP Conjugate. GAPDH Antibody-HRP Conjugate was diluted 1:200 in HRP Conjugate Diluent 15 min before addition to wells. After 1 h at 37 °C the assay plate was washed five times with 250 µl/well of 1x Wash Solution. 100 µL/well of TMB substrate were added and incubated for 25 min at room temperature. 100 µL/well of Stop Buffer were added to stop the reaction. The signal at 450 nm was detected on a VICTOR Nivo multimode plate reader equipped with filter-based absorbance detection. The absorbance detection protocol, pre-loaded in the instrument's software was adjusted to reflect the use of the 450 nm filter and a 100 ms flash time.

Results

The linear range of the assay was tested using the GAPDH standard provided in the kit. The results showed good linear correlation up to a GAPDH standard concentration of 600 ng/mL. The measured values are in line with the standard curve presented in the manufacturer's protocol, although low concentrations showed higher standard deviation (SD) (Fig.1).

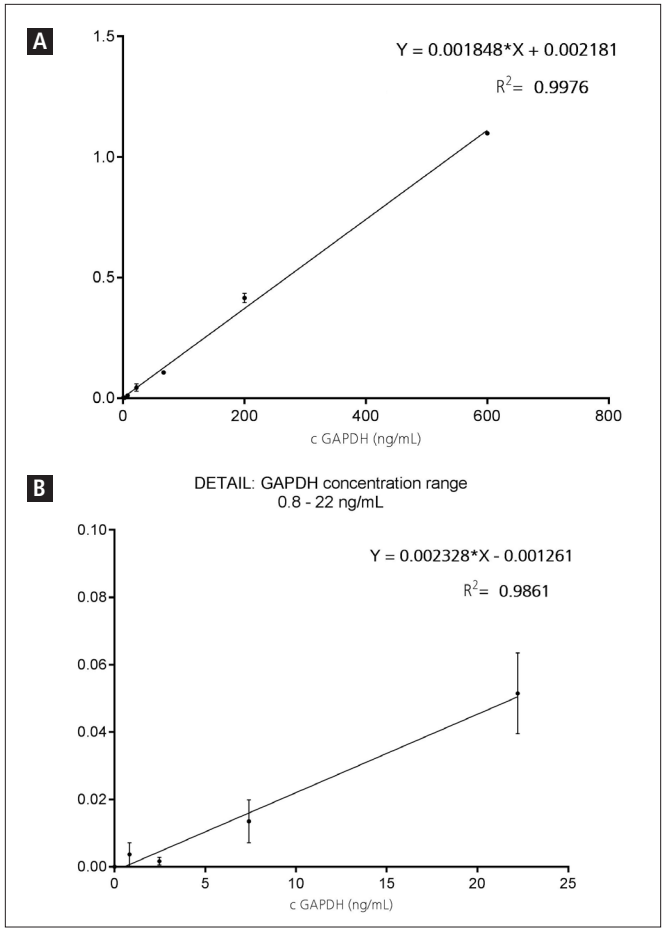

Figure 1. OD_{450} detection of GAPDH standard. A: Linear regression of the GAPDH standard dilution series in 96-well format. B: Linear regression of the GAPDH standard in the concentration range 0.8-22 ng/mL. Values represent the mean of three replicates ± SD.

Conclusion

This study confirms the suitability of the VICTOR Nivo multimode plate reader for ELISA assays. The measured dynamic range and assay linearity were in line with the MaxDiscovery GAPDH ELISA kit manual. The VICTOR Nivo system's software includes pre-written protocols which can be easily adjusted, if necessary, to suit the specific requirements of particular assays.

References

1. MaxDiscovery™ Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) ELISA Kit Manual, #3401, Bioo Scientific.

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