Measuring Enzyme Activity Using the Thermo Scientific NanoDrop 3300 Fluorospectrometer.

Application Note

Sandra Götze M.Sc.[1], Dr. Reinhard Saborowski[1], David Schlesinger Ph.D.[2]
[1] Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany
[2] Thermo Fisher Scientific, Madison, WI

Introduction

Thermo Scientific™ NanoDrop™ instruments have become the gold standard for the quantification and analysis of nucleic acids and proteins; however, they are well suited to a variety of other applications. The NanoDrop 3300 Fluorospectrometer, in particular, is a very versatile instrument.

Fluorophores have become a ubiquitous and powerful tool for life science researchers. They may be used as reporter groups of fluorogenic substrates needed for the determination of enzyme activities. These substrates are non-fluorescent in their initial "structure" and only through enzymatic cleavage have their fluorescent properties become apparent. Thereby, they are very sensitive and suitable to detect even very low enzyme activities. If fluorogenic substrates are designed for very specific research purpose, they are often very expensive or limited in quantity.

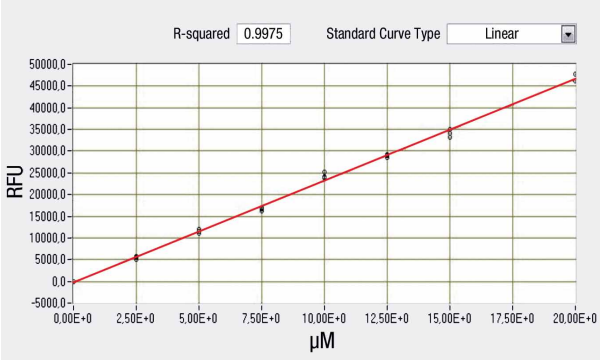
An instrument capable of using small volumes of fluorogenic substrates to measure enzyme activity is very practical. The NanoDrop 3300 has proven to be a useful tool in this regard, because of its microliter sample volume requirement. In addition, the capabilities of the NanoDrop 3300 software are ideally suited for measuring enzymatic activity. Here, we outline a simple and robust assay for measuring proteasomal activities in cell extracts.

Experimental Procedures

Reproducibility and linearity of fluorescent measurements were determined through the use of a standard curve created from serial dilutions (0 - 20 µM) of the fluorophore 7-amino-4-methylcoumarin (AMC). The excitation and emission spectra for AMC falls well within the range of the NanoDrop 3300, with an excitation wavelength of 365 nm and an emission wavelength of 437 nm.[2]

Enzyme activities of the 26s proteasome multi-enzyme complex were assayed in cell extracts from the hemolymph of the marine crab *Cancer pagurus*.[1] Specific proteasomal activity was determined through the use of fluorophore conjugated peptides. At regular intervals, 2 µl aliquots of reaction mixtures were measured with the NanoDrop 3300.

Fig. 1 Standard curve of increasing AMC concentrations generated with a Thermo Scientific NanoDrop 3300 Fluorospectrometer.



Thermo Scientific NanoDrop 3300 Fluorospectrometer

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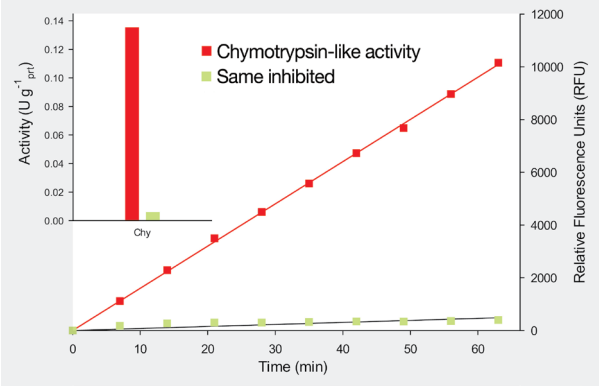
Results

The linearity of the AMC standard curve was excellent as shown in Fig. 1, with very consistent replicates indicating a high level of precision.

Fig. 2 Measurements of proteasomal activity measured with a Thermo Scientific NanoDrop 3300 Fluorospectrometer.



The enzymatic activity of the 26S proteasome produced linear results over time (Fig. 2) again showing the high level of reproducibility achievable with a NanoDrop instrument. At the higher AMC concentrations the NanoDrop 3300 outperformed a standard conventional fluorometer, (data not shown).

Conclusions

The NanoDrop 3300 was shown to be a very practical tool for the measurement of enzymatic activity using fluorogenic substrates. Due to the unique capability of the NanoDrop 3300 to measure very small reaction volumes, it is ideal for enzymatic assays requiring the use of fluorogenic substrates, enzymes or cofactors, which are costly and/or available in limited quantities.

References

1. Götze, S.; Saborowski R. *"NanoDrop fluorometry adopted for microassays of proteasomal enzyme activities" Anal. Biochem.* 2011, 413(2), 203-205.

2. PubChem Compound Identifier 92249

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