Assessing pancreatic trypsin activity using the microplate reader from BMG LABTECH

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- **Trypsin activity as a measure for acute pancreatitis monitored using a fluorimetric kinetic assay**
- **Microplate reader increases efficiency and productivity compared to conventional spectrophotometer measurements**

Introduction

Pancreatic trypsin activity is one of the commonly used biomarkers for assessing severity of both in vivo and in vitro experimental acute pancreatitis. Currently, it is measured by fluorimetric assay using a spectrophotometer in which samples are excited at 380 nm and emissions collected at 440 nm. Although this method provides an accurate measurement of trypsin activity, it only allows testing of individual samples per time point (about 5 mins) and requires a considerable amount of trypsin substrate which is expensive. The BMG LABTECH microplate reader offers the possibility to test pancreatic trypsin activity with a minimal volume of sample and dyes in multiwell format and in real time.

Assay Principle

Trypsin is a serine protease and is known to cleave peptide chains mostly at the carboxyl side of lysine or arginine. This property is used to determine the trypsin activity in a fluorescent assay. The known trypsin substrate Boc-Gln-Ala-Arg-MCA contains after the amino acid Arg (arginine) a fluorescent dye called MCA (7-methoxycoumarin-4-acetic-acid). After cleavage of substrate the MCA dye is set free and can be detected via fluorescence intensity. In this application note different ex/em settings were used to detect trypsin activity.

Materials & Methods

- normal and acute pancreas samples obtained from CD1 mice and C57BL/6 mice
- black 96w flat bottom microplates from Greiner
- Boc-Gln-Ala-Arg-MCA, Peptide, Osaka, Japan
- Microplate reader from BMG LABTECH

Preparation of pancreas samples

Mouse pancreata were homogenised by a motorised homogeniser on ice in tissue homogenisation buffer pH 6.5, containing (in mM) MOPS 5, sucrose 250 and magnesium sulphate. The resulting homogenates were centrifuged at 1500 g for 5 min, and 15 µL of each supernatant was added into a 96-well microplate.

Addition of trypsin substrate

Trypsin substrate Boc-Gln-Ala-Arg-MCA was added into assay buffer pH 8.0, containing (in mM) Tris 50, NaCl 150, $CaCl_2$ 1 and 0.1 mg/mL bovine serum albumin. The final working concentration for the substrate was 50 µM. The mixture (285 µL) was added into the microplate allowing the cleavage of trypsin by the substrate. The final volume in the well was 300 µL.

Detection of trypsin activity

The microplate reader was set in kinetic mode (plate mode). The samples were excited at 380 nm and emissions collected at 440 nm for a minimum of 5 mins. A standard curve was generated using commercially available porcine trypsin (Sigma) in different concentrations (0 – 150 µM). The fluorescence intensity at 3 mins for standards and samples was used for final analysis. The enzyme activity was calculated from the substrate conversion and related to the protein content in the well. To determine pancreatic protein concentration a BCA protein assay (Thermo, Rockford, USA) measuring absorbance at 562 nm was utilized.

Testing of different excitation and emission settings

The microplate reader was further used to test different excitation and emission settings for the determination of MCA. Next to the ex/em 380/440 combination an ex/em 355/440 combination was used and corresponding standard curves were obtained.

Results & Discussion

Detection of trypsin activity

For the porcine trypsin concentrations given, the microplate reader gave very accurate readouts (R2 = 0.9943) as shown in Fig.1. Pancreatic trypsin activity of samples from both normal and acute pancreatitis was accurately determined by the instrument. The results from the plate reader and the spectrophotometer (data not shown) are highly comparable (k = 0.9147).

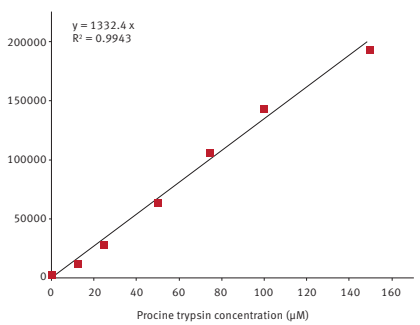

Fig. 1 Standard trypsin activity curve. Commercially available porcine trypsin was used as the trypsin standard in a concentration range of 0-150 µM.

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Testing several groups of samples using the spectrophotometer required a few hours of work but only required 10 minutes to finish the procedure on the plate reader, thus the instrument greatly increases efficiency and productivity. Moreover, only 1.5 µL of substrate was needed per sample in the microplate reader compared to 10 µL in the spectrophotometer.

Testing of different ex/em combinations

In the literature different excitation and emission wavelengths are used to determine the release of MCA in samples. Mostly the combination excitation/emission 380/440 nm or 380/460 are described although the excitation maximum of MCA is at a shorter wavelength. Because of that an Ex355/440 filter combination was tried as well. Figure 2 shows signal curves obtained for these two different ex/em combinations under identical conditions.

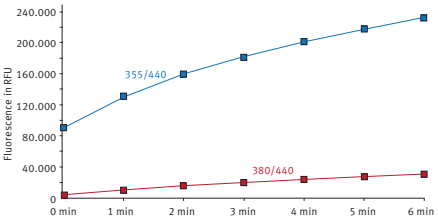
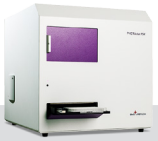


Fig. 2 Signal curves for 355/440 and 380/440 ex/em combination in a trypsin assay using Boc-Gln-Ala-Arg-MCA as substrate.

With the 355/440 ex/em combination higher relative fluorescence values are obtained compared to the conventional 380/440 ex/em wavelengths. But it is necessary to mention that also the blank value (RFU value at t = 0) is significantly higher. Based on these results a 355/440 could be preferred if only low signals are obtained.

Conclusion

Testing pancreatic trypsin activity in a BMG LABTECH microplate reader is quick and accurate. It allows for a much higher throughput of samples and has reduced the expense of costly consumables required for testing pancreatic trypsin in a spectrophotometer. Further, with this instrument it is very convenient to test different filter settings in a simple software-supported multichromatic mode.



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