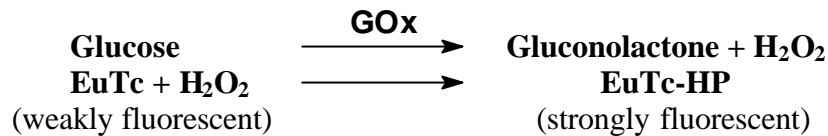


Application Note EuTc 004: Detection of Glucose or Glucose Oxidase by EuTc Reagent

Introduction

Glucose oxidase (GOx) is widely used for glucose determination. Chromeons EuTc-Reagent can be used for sensitive (10-fold more than o-dianisidine) optical detection of both glucose and glucose oxidase. The assay works at neutral pH and is easy to perform (4 solutions only). Fluorescence measurements can be carried out with a fluorescence microplate reader or fluorometer. Time resolved detection as well as fluorescence intensity based detection is possible.

Detection scheme:



Experimental Setup

Materials

The following solutions should be used:

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| <i>Solution A</i> (EuTc standard solution): | dissolve the contents of Chromeon's Eu-TC vial in 100 mL of distilled water. |
| <i>Solution B</i> (GOx stock solution): | Glucose oxidase (GOx, EC 1.1.3.4, from <i>Aspergillus niger</i>) is used without further purification and unit as defined by the provider). In the shown example GOx was from Sigma-Aldrich; concentration: 54.1 U/mL). |
| <i>Solution C</i> (glucose stock solution): | dissolve 1.250 g of glucose in 25 mL of MOPS buffer (solution D). The glucose stock solution is stored overnight before use to allow the equilibrium of α - and β -anomers. |
| <i>Solution D</i> (10 mM MOPS buffer): | dissolve 2.3 g of MOPS sodium salt in 800 mL of distilled water, add 1.0 M HCl to adjust the pH to 6.9, and make up the volume to 1000 mL with distilled water. |

Instrumentation

Fluorescence measurements were performed in top reading mode on a Genios+ (Tecan, Grödig, Salzburg, Austria) microtiter plate (MTP) reader.

Excitation filters:	405 nm
Emission filters	612 nm
For fluorescence intensity measurements:	lag time: 0 μ s.
For time-resolved (gated) detection:	lag time: 60 μ s; integration time: 40 μ s
Temperature:	30.0 \pm 0.1 $^{\circ}$ C.

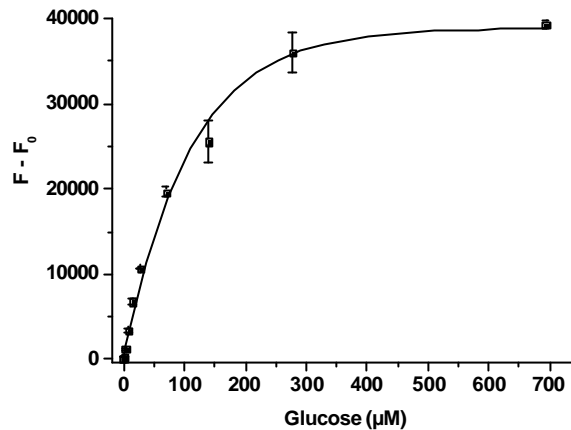
Protocol for Glucose:

100 μ L of *solution A*, 20 μ L of *solution B*, and 80 μ L of *solution D* are added in each well of a thermostated (30 $^{\circ}$ C) 96-well microtiter plate. The samples (50 μ L) containing glucose with a concentration between 5 and 100 μ M are added simultaneously, and the plates are detected under intervals of shaking.

Calibration solutions for glucose are obtained by dilution of *solution C* with *solution D* by a factor of 1:50 000 (= 5.5 μ mol), 1:25 000 (= 11 μ mol), 1:10 000 (= 27.7 μ mol), 1:5 000 (= 55.4 μ mol) and 1:2 500 (= 109 μ mol).

Control experiments are conducted by adding buffer in place of glucose samples, denoted as F_0 . The blank is subtracted from the fluorescence with glucose for the detection. Usually 30 minute incubation at 30 °C will facilitate the detection, especially for extremely low glucose concentrations.

Example of a calibration plot for glucose detection:



Protocol for Glucose Oxidase:

100 µL of *solution A*, 15 µl of *solution C*, and 85 µL of *solution D* are added in each well of a thermostated (30 °C) 96-well microtiter plate. The samples (50 µL) containing GOx with an activity between 1.5 and 15 mUnit/mL are added simultaneously, and the plates are detected under intervals of shaking.

Calibration solutions for GOx are obtained by dilution of *solution B* with *solution D* by a factor of 1:45 000 (= 1.2 mU/mL), 1:20 000 (= 2.7 mU/mL), 1:10 000 (= 5.4 mU/mL), 1:5000 (= 10.8 mU/mL), and 1:3500 (= 15.5 mU/mL).

Control experiments are conducted by adding buffer in place of GOx samples as blank and its intensity denoted as F_0 . The blank is subtracted from the fluorescence with glucose oxidase for the detection. Usually 30 minute incubation at 30 °C will facilitate the detection, especially for extremely low GOx concentrations.

Example of a calibration plot for GOx detection:

