

Measurement of GOD enzyme activity in conjugates

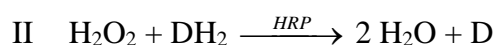
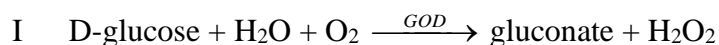
WOLF D. KUHLMANN

Division of Radiooncology, Deutsches Krebsforschungszentrum, D-69120 Heidelberg

Laboratory Diagnostics & Cell Science, 56112 Lahnstein

For all labeling purposes, highly purified glucose oxidase preparations from *Aspergillus niger* (GOD) are employed. The quantitative measurement of glucose oxidase follows the principle described by HU BERGMEYER (*Methods of Enzymatic Analysis*, Verlag Chemie, Weinheim-New York 1983). *

Principle



(DH₂ = Leuco-dye; D = dye)

Assay

Wavelength = 436 nm; light path = 1 cm; final volume = 3.0 mL; blank = phosphate buffer; temperature = 25°C; $\epsilon_{436 \text{ nm}} = 8.3 \text{ [cm}^2/\mu\text{mole]}$;

Pipette into cuvette:

2.47 mL of o-dianisidine solution (0.066 mg o-dianisidine·HCl/mL 0.1 mol/L phosphate buffer pH 7.0); buffer previously saturated with O₂ for 10 min
+ 0.50 mL D-glucose solution (100 mg/mL buffer)
+ 0.01 mL of HRP solution (2 mg HRP RZ 3.0 per mL buffer)

mix and start the reaction by adding of
0.02 mL sample (ca. 0.002 mg GOD/mL)

mix and read change in optical density per min: changes after 1, 2, 3, 4 and 5 min are measured; calculate $\Delta E/\text{min}$ (mean value).

Calculation

$$\text{Volume activity} = \frac{3.00}{8.3 \cdot 1.00 \cdot 0.02} \cdot \Delta E/\text{min} \text{ (U/mL sample)}$$

$$\text{Specific activity} = \frac{\text{volume activity}}{\text{concentration}}$$

* Chromogens and other chemicals can be toxic. They must be handled with care

Commercially available GOD from *Aspergillus niger* can possess a range of different specific activities. For labeling purposes, activities in the order of 210 U/mg are preferred.

References for further readings

- Müller D (1928)
Franke W and Lorenz F (1937)
Keilin D and Hartree EF (1952)
Keston AS (1956)
Hugett ASG and Nixon DA (1957)
Bodmann O and Walter M (1965)
Bright HJ and Appleby M (1969)
Kuhlmann WD and Avrameas S (1971)
Rogers MJ and Brandt KG (1971)
O'Malley JJ and Weaver JL (1972)
Tsuge H and Mitsuda H (1973)
Bergmeyer HU and Berndt E (1974)
Lott JA and Turner K (1975)
Tsuge H *et al.* (1975)
Bergmeyer HU, Bergmeyer J, Grassl M (eds.) in *Methods of Enzymatic Analysis* (1984)
Kuhlmann WD (1984)
Kunst A *et al.* (1984)

Full citation of publications is given in chapter *References*

link: <https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf>