Measurement of HRP enzyme activity in conjugates

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For all labeling purposes, highly purified peroxidase preparations from horseradish (HRP) with **RZ 3** are employed; Reinheitszahl RZ = $A_{403\,nm} : A_{275\,nm}$. These preparations are highly active, approx. 250 units/mg, measured with H_2O_2 and guaiacal.

For the quantitative measurement of peroxidase activity in the spectrophotometer the following method is used. *

Principle

$$H_2O_2 + DH_2 \xrightarrow{HRP} 2\,H_2O + D$$

(DH_2 = Leuco-dye; D = dye)

A large number of dyes exist for the measurement of peroxidase activitity. Here, we give a widely employed procedure.

Assay

Wavelength = 436 nm; light path = 1 cm; final volume = 3.11 mL; blank = phosphate buffer; $\varepsilon_{436\,nm}$ = 6.39 [cm^2/µmole]; temperature = 25ºC;

Δ E/min (change in optical density in the spectrophotometer at 436 nm per unit time) is used to calculate peroxidase activity.

Pipette into cuvette:

3.00 mL of 0.1 mol/L phosphate buffer pH 7.0
\+ 0.05 mL guaiacol (245 mg/100 mL distilled water)
\+ 0.02 mL sample (ca. 0.002 mg of HRP/mL)

mix and start the reaction by adding
0.04 mL H_2O_2 solution (1.0 µL of 30% H_2O_2/mL phosphate buffer)

mix and read change in optical density per min: when the extinction is between E = 0.2 and E = 0.25, then read change after 1, 2 and 3 min and calculate Δ E/min (mean value).

Calculation

$$\text{Volume activity} = \frac{3.11}{6.39 \cdot 1 \cdot 0.02} \cdot \Delta\ \text{E/min (U/mL sample)}$$

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

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

Specific activity of HRP RZ 3.0 is approximately 250 U/mg lyophilized enzyme measured with guauacol; U/mg protein, protein determination according to LOWRY et al. (1951).

References for further readings

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Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

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