

Measurement of HRP enzyme activity in conjugates

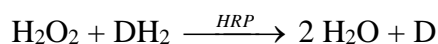
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For all labeling purposes, highly purified peroxidase preparations from horseradish (HRP) with **RZ 3** are employed; Reinheitszahl RZ = $A_{403 \text{ nm}} : A_{275 \text{ nm}}$. These preparations are highly active, approx. 250 units/mg, measured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

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

Principle



(DH_2 = Leuco-dye; D = dye)

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

Assay

Wavelength = 436 nm; light path = 1 cm; final volume = 3.11 mL; blank = phosphate buffer; $\epsilon_{436 \text{ nm}} = 6.39 [\text{cm}^2/\mu\text{mole}]$; temperature = 25°C;

$\Delta E/\text{min}$ (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% $\text{H}_2\text{O}_2/\text{mL}$ 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 $\Delta E/\text{min}$ (mean value).

Calculation

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

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

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

Specific activity of HRP RZ 3.0 is approximately 250 U/mg lyophilized enzyme measured with guaiacol; 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.immunologie-labor.com/cellmarker_files/References.pdf