Determination of the RZ Value for PEROXIDASE

CONDITIONS: T = 25° C, A_{403nm} and A_{275nm}, Light path = 1 cm

METHOD: Spectrophotometric

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 6.0 at 25°C
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.0 at 25°C with 1 M KOH.)

B. Peroxidase Enzyme (POD)
(Immediately before use, prepare 10 ml of a solution containing approximately 0.3 mg solid/ml of Peroxidase in Reagent A. Gently mix by inversion until the solids are completely dissolved.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes[1]:

	Test	Blank
Reagent B (POD)	3.00	-------
Reagent A (Buffer)	------	3.00

Record the absorbance at 403 nm and 275 nm for both the Test and Blank using a suitable spectrophotometer.[2]

CALCULATION:

$$RZ = \frac{(A_{403nm}\ \text{Test} - A_{403nm}\ \text{Blank})}{(A_{275nm}\ \text{Test} - A_{275nm}\ \text{Blank})}$$

RZ = Reinheitszahl, A_{403}/A_{275} value

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REFERENCE:

Shannon, L. M., Kay, E. and Lew, J. Y. (1966) *Journal of Biological Chemistry*, 241, 2166-2172

NOTES:

1. The Test and Blank should be done in duplicate.
2. The difference in absorbance due to the cuvettes at 403 nm and 275 nm must be accounted for in the calculation of RZ.
3. The RZ determination is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.