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ASSAY / ANALYTICAL PROCEDURE
ASCORBATE OXIDASE

1. METHOD OF ASSAY:

Based on that of Oberbacher and Vines in which the decrease of absorbance due to oxidation of ascorbate by AO (Ascorbate Oxidase) is measured at 265nm and 25^{O}C.

$$2\text{L-ascorbate} + O_2 \xrightarrow{AO} 2\ \text{dehydroascorbate} + 2H_2O$$

2. UNIT DEFINITION:

That amount of enzyme catalysing the oxidation of 1 micromole ascorbate per minute at 25^{O}C.

3. REAGENTS:

3.1 **0,1M Phosphate / EDTA Buffer pH5,6**

Dissolve 12,84g KH_2PO_4 [MM 136,09], 0,735g Na_2HPO_4 [MM 141,96] and 0,186g EDTA. $Na_2.2H_2O$ [MM 372,24] in 800ml distilled H_2O, check pH5,6 (1N HCl or 1N NaOH), dilute to 1 000ml with distilled H_2O and recheck pH.

3.2 **Substrate [0,005M Ascorbic Acid]**

Dissolve 4,4mg L-ascorbic acid [MM 176,13] in 5ml ice-cold distilled H_2O. Store on ice. (PREPARE FRESH DAILY)

3.3 **Enzyme**

Dissolve 1 - 5mg enzyme / ml in buffer [3A]. Immediately before assay, dilute to yield $0{,}185 \leq u/ml < 0{,}278$ ($0{,}08 \leq \Delta A_{265}/min \leq 0{,}12$)

4. PROCEDURE:

Into 10mm quartz cells pipette the following at 25^{O}C:

	Blank	Test
Buffer [3.1]	3,0 ml	2,9 ml
Substrate [3.2]	0,1 ml	0,1 ml
Enzyme [3.3] at zero time	-	0,1 ml
	3,1 ml	3,1 ml

Place blank cell (PREPARE A FRESH BLANK FOR EACH ASSAY) in sample compartment, test cell in reference compartment and monitor absorbance over ± 3 minutes.

Record apparent increase of absorbance at 265nm for ± 4 minutes.

5. CALCULATION:

$$\text{Activity (u/mg)} = \frac{\dfrac{\Delta A_{265}/\text{min} \times 3{,}1 \times \text{dilution}}{13{,}386 \times 0{,}1}}{\dfrac{u/ml}{\text{mg enzyme / ml original solution}}}$$

[ε: 13,386; cuvette volume: 3,1ml; enzyme volume: 0,1ml]

6. BIBLIOGRAPHY:

Oberbacher M.F. and Vines H.M,: 1963 Nature 197 1203.