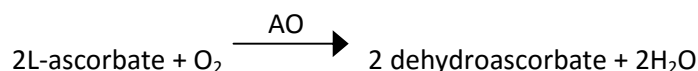


ASSAY / ANALYTICAL PROCEDURE
ASCORBATE OXIDASE

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°C.



2. UNIT DEFINITION:

That amount of enzyme catalysing the oxidation of 1 micromole ascorbate per minute at 25°C.

3. REAGENTS:

3.1

0,1M Phosphate / EDTA Buffer pH5,6

Dissolve 12,84g KH₂PO₄ [MM 136,09], 0,735g Na₂HPO₄ [MM 141,96] and 0,186g EDTA. Na₂.2H₂O [MM 372,24] in 800ml distilled H₂O, check pH5,6 (1N HCl or 1N NaOH), dilute to 1 000ml with distilled H₂O and recheck pH.

3.2

Substrate [0,005M Ascorbic Acid]

Dissolve 4,4mg L-ascorbic acid [MM 176,13] in 5ml ice-cold distilled H₂O. Store on ice. (PREPARE FRESH DAILY)

3.3

Enzyme

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

4. PROCEDURE:

Into 10mm quartz cells pipette the following at 25°C:

	<u>Blank</u>	<u>Test</u>
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
	<u>3,1 ml</u>	<u>3,1 ml</u>

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

5. CALCULATION:

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

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

6. BIBLIOGRAPHY:

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