**Lab Sheet 2**

# **Lab (2): Quantitative amino acids estimation by ninhydrin method**

**Objectives:**

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**Protocol:**

1. Set up 7 test tubes as following:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tube  | Standard amino acid solution (100 µg/ml) (ml)  | Distilled water (ml)  | Unknown sample  | Ninhydrin reagent (ml)  |
| **Blank**  | -  | 4  |   | 1  |
| **A**  | 1.2  | 2.8  |   |
| **B**  | 1.6  | 2.4  |   |
| **C**  | 2  | 2  |   |
| **D**  | 2.4  | 1.6  |   |
| **E**  | 2.8  | 1.2  |   |
| **Sample with Unknown concentration** | -  | -  | 4  |

1. Mix the contents of the tubes by vertexing/shaking the tubes.
2. Cover the mouth of the tubes with aluminium foil.
3. Place all the test tubes in 80 ℃ for 15 minutes.
4. Cool the test tubes in cold water, the add 1 ml of ethanol to each test tube and mix well.
5. Record the absorbance of all tubes against the blank at 570 nm using a colorimeter (spectrophotometer).

 PAUSE AND THINK ➔ What the blank should contain? Why?

1. Calculate the amino acid concentration for each standard amino acid solution using C1 x V1 = C2 x V2 formula.
2. Plot standard curve for absorbance against amino acids concentration (µg/ml) using results for solutions (A-E).
3. From the standard curve, estimate the concentration of the amino acids present in your unknown sample.

 **Results:**

|  |  |  |
| --- | --- | --- |
| **Test tube**  | **Amino acid concentration** **[µg/ml]**  | **Absorbance at 570 nm**  |
| **Blank**  |   |   |
| **A**  |   |   |
| **B**  |   |   |
| **C**  |   |   |
| **D**  |   |   |
| **E**  |   |   |
| **Sample with Unknown concentration** | \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  |   |

**Discussion:**

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