**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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