**ESTIMATION OF TOTAL FREE AMINO ACID – NINHYDRIN METHOD**

**INTRODUCTION:**

The amino acids are colorless ionic compounds that form the basic binding of protein. Apart from being bound as protein, amino acids exist in the free form in many tissues and also known as free amino acids. They are mostly water soluble in nature. They occur very often in plants during disease condition. The free amino acid composition exhibit a change and hence the measurement of that free amino acid gives the physiology and health status of plants.

**AIM:**

To estimate the amount of free amino acid present in 100ml of the given sample.

**PRINCIPLE:**

Ninhydrin, a powerful oxidizing agent decarboxylates the α amino acid and yields an intensely coloured bluish purple product which is colorimetrically measured at 570nm.

Ninhydrin + α amino acid → Hydrindantin + decarboxylated amino acid + CO2 + NH3

Hydrindantin + Ninhydrin + NH3→ Purple coloured Product + H2O

**MATERIALS REQUIRED:**

**1. NINHYDRIN:**

Dissolved 0.8g stannous Chloride (Sncl2.2H2O) in 500ml 0.2m citrate buffer. pH 5.0. Add the solution to 20g of Ninhydrin in 500 ml of methyl (methoxy ethanol)  
 0.2m citrate buffer pH 5

**2. DILUENT SOLVENT:**

Mix equal volume of Water and n-Propanol and use.

**3. STANDARD:**

Dissolve 50 mg of leucine in 50ml distilled water in a volumetric flask. Take 10ml of stock standard and dilute to 100 ml in another volumetric flask for working standard solution.

**4. EXTRACTION OF AMINO ACIDS:**

Weighed 500 mg of plant sample and grind it in a mortar and pestle with a small quantity of acid. Washed and to this homogenate added 5-10ml of 80% ethanol. Filtered or centrifuged and saved the filterate or the supernatant. Repeat the extraction twice with the residue and pool all the supernatant. Reduce the volume needed by evaporation and use the extract for the quantitative estimation of total free amino acid. If the tissue is tough use boiling 80% ethanol for extraction.

**PROCEDURE:**

1. Into a series of test tubes pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0ml of working standard solution which gives a concentration range of 20, 40, 60, 80, 100µg.

2. Prepared the reagent blank by taking 2ml distilled water or by taking 0.1ml of 80% ethanol instead of the extract.

3. Taken 0.5ml of extract as test and the volume was made upto 2ml with distilled water

4. To all the test tubes added 1ml of Ninhydrin solution.

5. To all the test tubes added 5ml of diluent and mixed the contents.

6. Heated the tubes in boiling water bath for 20 minutes.

7. After 15 minutes read the intensity of purple colour against the reagent blank in a colorimeter at 590nm.

8. Draw a standard graph by taking the concentration on X axis and optical density on Y axis. From the graph, calculate the concentration of total free amino acid in the sample and express as percentage equivalence of leucine.

**RESULT:**

The Concentration of total free amino acid present in the given unknown sample was found to be \_\_\_\_\_\_\_\_ mg

**STOCK STANDARD SOLUTION:** Concentration: 1mg/ml, 50 mg of leucine in 50 ml of distilled water.

**WORKING STANDARD SOLUTION:** Concentration: 100µg/ml, 10ml of the stock made upto 100ml with distilled water.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **PARTICULARS** | **B** | **S1** | **S2** | **S3** | **S4** | **S5** | **T** |
| 1 | Volume of working standard (ml) | - | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | - |
| 2 | Concentration of working standard (µg) | - | 20 | 40 | 60 | 80 | 100 | - |
| 3 | Volume of Unknown (ml) | - | - | - | - | - | - | 0.5 |
| 4 | Volume of Water  (ml) | 2.0 | 1.8 | 1.6 | 1.4 | 1.2 | 1.0 | 1.5 |
| 5 | Volume of Ninhydrin(ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Heat the contents of the test tube in a boiling water bath for 20 minutes | | | | | | | | |
| 6 | Volume of Diluent (ml) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Kept the test tubes at a room temperature for 15 minutes. The Purple color developed was read at 570nm | | | | | | | | |
| 7 | Optical Density at 570 nm |  |  |  |  |  |  |  |

**CALCULATION:**

Optical density \_\_\_\_\_\_\_\_\_ corresponds to \_\_\_\_\_\_ µg of free amino acid

0.5 ml of unknown corresponds to \_\_\_\_\_ µg of free amino acid

100ml of unknown contains \_\_\_\_\_ x100/1000

= \_\_\_\_\_\_ mg of amino acid

The amount of free amino acid present in the given sample = \_\_\_\_\_ mg