Proteomic techniques such as two-dimensional electrophoresis can be used to study the entire proteome i.e. all the protein content of cells.

Learning Objectives:

After interacting with this learning object, the learner will be able to:

- Perform the experiment to test the presence of amino acid in the sample.
- Identify the mechanism involved in the detection.
- Operate the steps used in colorimeter.
- Infer the law governing the colorimetric analysis.
- Assess the troubleshooting steps involved in the experiments.

Note: The current IDD exists in two modes - interactive and automatic. Students taking lab course should select interactive (set as default), while the automatic mode may be selected for general users.
Ninhydrin Reaction

α-amino acid reacts with excess of Ninhydrin reagent to give purple color and the intensity of the color is directly proportional to the concentration of amino acid.
Beer-Lambert’s Law

Colorimeter works on the basis of Beer-Lambert’s law; the law relates the absorbance and the concentration of the solution. In the equation, $L$ signifies the path length, $C$ concentration, $\varepsilon$ (epsilon) absorption coefficient and $I_0$ and $I$ corresponds to the intensity of light before entering the solution and the intensity of emergent light from the solution, respectively.

$$\log_{10} \frac{I_0}{I} = \varepsilon L c$$

Greek letter, epsilon

Concentration of the solution (mol dm$^{-3}$)

Length of solution the light passes through (cm)
Reagents of Ninhydrin Test

Clean the surface of the balance. Place a butter paper on the balance and tare the weight.
Reagents of Ninhydrin Test

Prepare stock solution of amino acid with concentration 1mg/ml.
Reagents of Ninhydrin Test

Prepare ninhydrin solution by dissolving weighed ninhydrin in ethanol as per required volume.
Reagents of Ninhydrin Test

Prepare 50% ethanol containing 50ml of water and 50ml of Ethanol.
Ninhydrin Test

Wash the boiling tubes; label the tubes as blank, standards as 0.2, 0.4, 0.6, 0.8 and 1ml and unknown 1,2, 3.
Ninhydrin Test

Prepare the standard solution of amino acid of varying concentration by taking in different volumes from the stock solution of 1mg/ml and then unknown sample.
Ninhydrin Test

Make the volume to 4ml using the distilled water in all the tubes.
**Ninhydrin Test**

Add 1000µl of ninhydrin reagent in all the tubes and mix well.
Ninhydrin Test

Cover the tubes with aluminum foil and place them in boiling water bath.
Ninhydrin Test

Place the tubes in the boiling water for 15 minutes. This condition initiates the reaction between ninhydrin and amino acids.
Ninhydrin Test

Place the tubes in cold water and allow it to cool down. Add 1 ml of 50% ethanol in all the tubes.
Calometric Analysis

Switch on the colorimeter and set the wavelength to 570nm to take the absorbance.
Calometric Analysis

Rinse the cuvette with the blank and discard it. Fill the cuvette with the blank solution and take the OD, auto zero the instrument and take the readings for all the other tubes.
Calometric Analysis

Plot the graph between OD at 570nm and the concentration of the sample and extrapolate the unknown OD value to find the concentration.
Definitions of the components/Keywords

Quantitative and Qualitative Estimation of Amino Acids-Ninhydrin Test

1. Amino acid: The basic monomeric unit of polypeptides and proteins. There are twenty standard amino acids with different structures and properties that can be combined in multiple ways to make up the wide range of proteins known to us. Each amino acid is also specified by a three-letter and single letter code.

2. Ninhydrin: Ninhydrin (2,2-Dihydroxyindane-1,3-dione), used to detect ammonia in primary and secondary amines. During the reaction with amines, purple colour known as Ruhemann’s purple is formed.

3. Beer-Lambert’s law: The law relates the absorbance and the concentration of the solution

\[
\log \frac{I}{I_0} = \varepsilon lc
\]

In the equation \( L \) signifies the path length, \( C \) concentration, \( \varepsilon \) (epsilon) absorption coefficient and \( I_0 \) and \( I \) corresponds to the intensity of light before entering the solution and the intensity after coming out of the solution. The intensity of the light coming out of the cuvette decreases when the concentration of the substances in the cuvette increases.
Amino acid can be detected using

- Chloroform
- Ethanol
- Acetone
- Ninhydrin

I am sorry, the correct answer is Ninhydrin.
Amines when react with Ninhydrin gives Purple color.

I am sorry, the correct answer is Purple color.
Absorbance can be measured using

- Calorimetry
- Colorimetry
- Spectrometry
- Refractometry

Congratulations, you have chosen the correct answer.
Colorimetry works based on

- Beers Law
- Lamberts Law
- Beer-Lamberts Law
- Raman spectrum

Congratulations, you have chosen the correct answer.
As the absorbance increases, the intensity of the outgoing light

- Decreases (Correct)
- Increases (Incorrect)
- Remains same (Incorrect)
- Zero (Incorrect)

I am sorry, the correct answer is Decreases.
References

Papers:


Links:

1) http://www.youtube.com/watch?v=JdXbTWf0c18

Books:


3) Biochemistry by Stryer et al., 5th edition.


5) Biochemistry by Voet & Voet, 3rd edition