

HiPer[®] Protein Estimation Teaching Kit (Qualitative)

Product Code: HTBC004

Number of experiments that can be performed: 25

Duration of Experiment: 3 hours

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Standard protein 1 and 2, Biuret and Ninhydrin reagents, Protein sample 1 and 2 at 2-8⁰C
- Other kit contents can be stored at room temperature (15-25⁰C)

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Aim:

To identify and characterize the different types of proteins by commonly used qualitative methods:

1. **Biuret Test**
2. **Ninhydrin Test**
3. **Xanthoproteic Test**
4. **Sulphur Test**
5. **Neumann's Test**

Introduction:

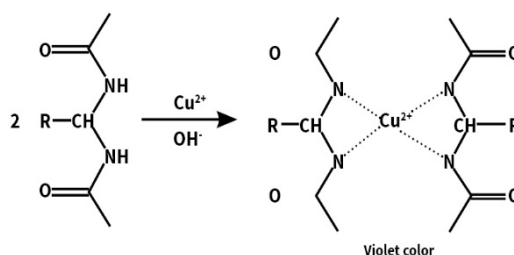
Proteins are polymers of amino acids. They are complex organic compounds containing nitrogen, hydrogen, carbon and oxygen. Proteins are abundant in our everyday food e.g. egg, soya bean, pulses, fish, milk etc. Presence of proteins can be confirmed qualitatively by several tests. Due to the presence of characteristic side chains in them, certain amino acids exhibit typical colour reactions that form the basis for their identification. In this kit, the following tests will be performed for the qualitative analysis of proteins.

1. Biuret Test
2. Ninhydrin Test
3. Xanthoproteic Test
4. Sulphur Test
5. Neumann's Test

Principle:

1. **Biuret Test:**

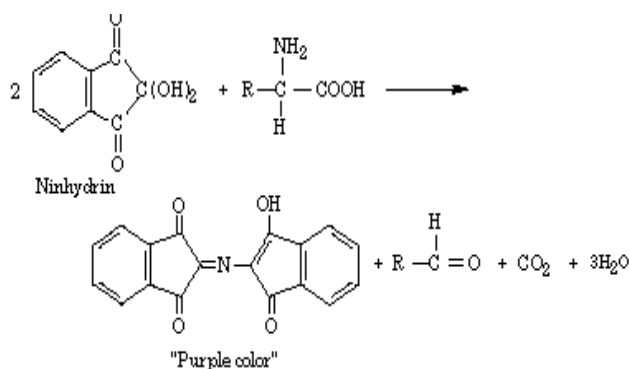
The Biuret test for proteins qualitatively detects the presence of proteins in solution with the development of a deep violet colour. In alkaline condition, Biuret ($\text{NH}_2\text{-CO-NH-CO-NH}_2$) reacts with compounds containing two or more peptide bonds to form complexes of violet colour. The name of this test comes from the name of the compound, Biuret which gives this test.



Biuret Reaction

2. **Ninhydrin Test:**

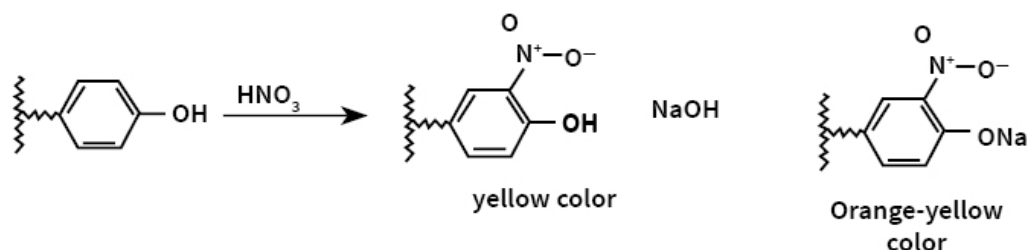
Amino acids contain a free amino and carboxyl group which react together with ninhydrin to produce a coloured product. In this reaction first, an amino group is attached to the first or alpha carbon of the amino acid's carbon chain and then the nitrogen atom of the amino group reacts with ninhydrin to give a blue-purple product. Amino acids that have secondary amino group attachments also react with ninhydrin and forms yellow coloured product. Proteins contain free amino groups on the alpha carbon and react with ninhydrin to form blue-violet compound called Rhamann's purple.



Ninhydrin Reaction

3. Xanthoproteic Test:

The Xanthoproteic test utilizes a nitration reaction which identifies the presence of activated benzene ring. The end product of this reaction is yellow in colour and the name comes from Greek word 'Xanthos' meaning yellow. Aromatic groups of either the free amino acid or protein undergo nitration on heating with concentrated nitric acid (HNO₃) and forms yellow coloured product. The salts of these derivatives are orange in colour.



Xanthoproteic Reaction

4. Sulphur Test:

Sulphur is present in proteins as cystine, cysteine or methionine. In alkaline media, lead acetate reacts to form black or brown coloured precipitate.

5. Neumann's Test:

Neumann's test is specifically used for casein. On heating with concentrated nitric acid and concentrated sulphuric acid, casein is digested and phosphorous is released. NH₃ is added to provide alkaline media. Ammonium molybdate reacts with NH₃ in alkaline media and forms yellow precipitate of phospho-ammonium molybdate.

Kit Contents:

The kit can be used to perform qualitative analysis of proteins.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			25 expts	
1	TKC257	Standard Protein 1(BSA)	115 ml	2-8°C
2	TKC258	Standard Protein 2 (Casein)	115 ml	2-8°C
3	TKC259	Standard Protein 3 (Gelatin)	115 ml	RT
4.	TKC255	Protein Sample 1	115 ml	2-8°C
5.	TKC256	Protein Sample 2	115 ml	2-8°C

6.	TKC251	Biuret Reagent	27 ml	2-8°C
7.	TKC252	Ninhydrin Reagent	27 ml	2-8°C
8.	TKC225	1 M NaOH	85 ml	RT
9.	TKC253	Lead acetate Solution	135 ml	RT
10.	TKC254	Ammonium molybdate solution	135 ml	RT

Materials Required But Not Provided:

Glasswares: Test tubes, Beakers

Reagents: Distilled water*, Concentrated Sulphuric acid, Concentrated Nitric acid

Other requirements: Test tube holder, Test Tube stand, Bunsen burner, Boiling water Bath, Pipettes/Dropper

*Molecular biology grade water is recommended (Product code: ML064)

Storage:

HiPer® Protein Estimation Teaching Kit (Qualitative) is stable for 12 months from the date of manufacture without showing any reduction in performance. Read Important Instructions before starting the experiment. Store all the reagents as specified in the brochure.

Important Instructions:

1. Read entire procedure carefully before starting the experiment.
2. Always use clean and dry glasswares.
3. Standard protein samples provided are Standard Protein 1, Standard Protein 2 and Standard Protein 3 (BSA, Casein and Gelatin).
4. Protein samples 1 and 2 are provided as test samples.
5. Perform all the experiments very carefully as the concentrated acids are very hazardous.

Procedure:

Read the important instructions before starting the experiment.

NOTE: Take 5 test tubes and label it as Standard Protein 1, Standard Protein 2, Standard Protein 3, Protein sample 1 and 2, individually for all the below mentioned tests.

A) Biuret Test:

1. Take 1 ml of each protein solution in separate test tubes.
2. Add 0.2 ml of 1 M NaOH solution to make it alkaline.
3. Add 200 µl of Biuret reagent.

B) Ninhydrin Test:

1. Add 1 ml of each standard and protein samples to separate test tubes.
2. Add 200 µl of Ninhydrin reagent and boil for until violet to dark blue color appears.
Note: Standard Protein 3 make take more time for color development (Boil until the color appears).

C) Xanthoproteic Test:

1. To 1 ml of each standard and protein sample, add 1 ml of concentrated Nitric acid and boil for 1 minute and allow it to cool to room temperature.
2. To the above solution add few drops (0.2 ml) of 1 M NaOH.

D) Sulphur Test:

1. To 1 ml of each standard and protein sample, add 1 ml of lead acetate solution. Mix well.
2. Add 0.2 ml of 1 M NaOH solution dropwise till precipitate dissolves. Boil for 1 minute.

E) Neumann's Test:

1. Take 200 µl of each standard and protein sample in separate test tubes.
2. Add 2 ml of concentrated nitric acid and 200 µl of concentrated sulphuric acid.
3. Boil till the volume reduces to 0.5 ml.
4. Allow it to cool down to RT and then add 1 ml of Ammonium molybdate solution

Observation and Result:

Table 2: After performing each test, record the results of the standard and test proteins as in the following table

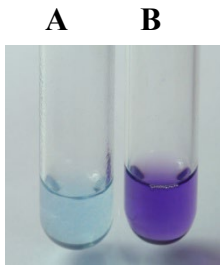
Sr. No	Test	Standard protein 1	Standard protein 2	Standard protein 3	Protein Sample 1	Protein Sample 2
1	Biuret Test	+	+	+		
2	Ninhydrin Test	+	+	+		
3	Xanthoproteic Test	+	+	-		
4	Sulphur Test	+	-	-		
5	Neumann's Test	-	+	-		

+: Positive result i.e. presence of protein

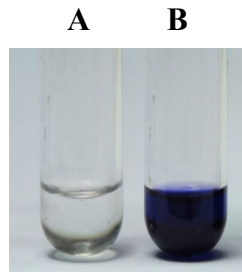
-: Negative result i.e. absence of protein

Positive result is indicated by the following observations:

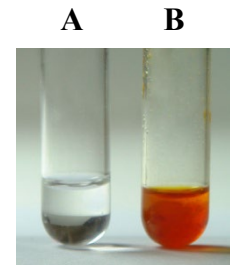
1. **Biuret Test:** The formation of violet coloured product
2. **Ninhydrin Test:** The formation of dark blue color
3. **Xanthoproteic Test:** The formation of yellow colour and orange coloured precipitate
4. **Sulphur Test:** The formation of brown or black colour
5. **Neumann's Test:** The formation of canary yellow precipitate



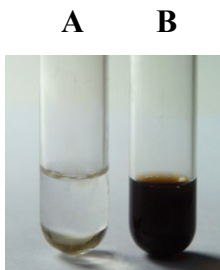
Biuret Test



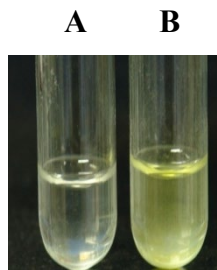
Ninhydrin Test



Xanthoproteic Test



Sulphur Test



Neumann's Test

A: Negative Control

B: Positive Test

Interpretation:

The different types of proteins are identified by the characteristic colour observed after performing each test and characterized by using various qualitative methods of protein estimation.









Troubleshooting Guide:

Sr.No	Problem	Possible Cause	Solution
1	False positive or false negative results	Procedure was not carried out properly	Follow the entire procedure carefully as mentioned above

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of Technical assistance mail at mb@himedialabs.com.

Symbol:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIHTBC004

Rev No.: 07

Date of Issue: 2025-08