



Millon's test:

Objective:

Object: to detect amino acid containing phenol group (hydroxyl group attached to benzene ring) Tyrosine give positive with Millon's test.

Principle of Millon's test:

Compounds containing hydroxyl benzene such tyrosine react with Million's reagent to form red complexes. Thus, this test is specific for the amino acid tyrosine and the protein containing this amino acid. Tyrosine when reacted with acidified mercuric sulphate solution gives yellow precipitate of mercury-amino acid complex. On addition of sodium nitrate solution and heating, the yellow complex of mercury-amino acid complex converts to mercury phenolate to red color.

Reagents:

test solution: 1 % arginine, 1 % tyrosine, phenol solution

(Millon's reagent (Acidified mercuric sulphate

sodium nitrite %1



ACTIVITY 2A Qualitative Tests for Proteins

- **Millon's Test**

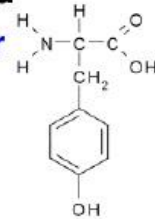
1 mL egg albumin + 2 drops **Millon's reagent**



mix and heat

(+) flesh precipitate to red color

- **Millon's reagent: mercurous nitrate in nitric acid**
- **Protein is precipitated as mercury salt and after heating precipitated turns flesh to red color**
- **Due to the phenol group contained in tyrosine**



Assistant professor



Procedure of Millon's test

.Take 1ml test solution in dry test tube

.Similarly, take 1ml distilled water in another test tube as control

.Add 1ml of Millon's reagent and mix well

.Boil gently for 1 minute

.Cool under tap water

.Now add 5 drops of 1 % sodium nitrite

.Heat the solution slightly

Look for the development of brick red precipitate

Result interpretation:

- **Positive Millon's test:** Brick red color (Tyrosine and phenol solution)
- **Negative Millon's test:** no red color (arginine)



❖ Millon's Test

➤ When Millon's reagent is added to a protein, a white precipitation is formed, which turns **brick red** on heating

➤ Phenols and phenolic compounds, when mixed with $\text{Hg}(\text{NO}_3)_2$ in nitric acid and traces

16

Assistant professor