**Experiment: 2**

**Color Tests for Proteins and Amino Acids**

**B)Color Test for Specific Amino Acids**

**1/Millon's Test:**

\*Used for detecting the presence of monohydroxybenzene derivatives (e.g: Tyrosine, Tyrosine derivatives, Phenol).

\*Millon's reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids.

\*Phenolic group of tyrosine ( OH ) reacts with Millon's reagent and give a red color, which is due to a mercury salt of nitrated tyrosine.

**Procedure:**

1-Take seven separate test tubes. Add 2 ml of: water, 1% egg albumin, 0.02% salicylic acid, 0.02% tyrosine, 1% gelatin, 0.02% phenylalanine and 0.02% phenol, respectively.

2-Add 3 drops of Millon's reagent to each tube.

3-Incubate in boiling water bath for 2 minutes. Note the color formed.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 0.02% phenol | 0.02% phenylalanine | 1% gelatin | 0.02% tyrosine | 0.02% salicylic acid | 1% egg albumin | H2O |
| 2 ml | 2 ml | 2 ml | 2 ml | 2 ml | 2 ml | 2 ml |
| Millon's Reagent | | | | | | |
| 3 drops | 3 drops | 3 drops | 3 drops | 3 drops | 3 drops | 3 drops |

Incubate in boiling water bath for 2 min. Not the color formed

**2/Hopkins-Cole Test:**

\*This test for Tryptophan.

Tryptophan + Hopkins-Cole reagent purple ring

\*Hopkins-Cole reagent contain magnesium glyoxylate (Magnesium powder and Oxalic acid).

\*Purple ring is formed by the reaction of glyoxylic acid ( CHO-COOH ) with the indole ring of tryptophan in presence of H2SO4.

**Procedure:**

1-Take six separate test tubes. Add 2 ml of: water, 1% egg albumin, 1% gelatin, 1% casein, 0.02% tryptophan and 0.02% tyrosine, respectively.

2-Add 3 ml of Hopkins-Cole reagent to each tube and mix.

3-Carefully add 5 ml of con. H2SO4 from buret by touching the tip of the stopcock to the inside wall of the test tube and allowing the acid to slowly drain down the tube so that the two liquids form separate layers. Add 5 ml of con. H2SO4 to each tube. Observe the color at the zone of contact of the two fluids. If no color appears, swirl the tube gently, but do not mix.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 0.02% tyrosine | 0.02% tryptophan | 1% casein | 1% gelatin | 1%egg albumin | H2O |
| 2 ml | 2 ml | 2 ml | 2 ml | 2 ml | 2 ml |
| Hopkins-Cole Reagent | | | | | |
| 3 ml | 3 ml | 3 ml | 3 ml | 3 ml | 3 ml |
| Concentrated H2SO4 | | | | | |
| 5 ml | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml |

Observe the color formed at the junction of the two liquids.

If necessary, gently rotate the tube to develop the colored ring.

**3/Sakaguchi Test:**

\*This test is specific for the guanidine group of arginine (H2N-C-NH), but a non-amino acid like creatine which contain this group will also answer this test.

\*This test positive for all proteins containing arginine and for arginine itself.

\*When arginine reacts with α-Naphthol and Sodium hypobromite (NaOBr) a red color result.

\*Red color is due to a reaction between the hypobromite and the –NH2 group of the guanidino part of arginine.

**Procedure:**

1-Take five separate test tubes. Add 5 ml of: water, 0.1% gelatin, 0.02% arginine, 0.02% creatine and 0.02% urea, respectively.

2-Add 1 ml of 10% NaOH to each tube.

3-Add 1 ml of 0.02% α-Naphthol to each tube.

4-Incubate all the tubes at room temperature for 3 minutes.

5-Add 4 drops of NaOBr to each tube. Note the color formed.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 0.02% urea | 0.02% creatine | 0.02% arginine | 0.1% gelatin | H2O |
| 5 ml | 5 ml | 5 ml | 5 ml | 5 ml |
| 10% NaOH | | | | |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| 0.02% α-Naphthol | | | | |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| Mix, incubate at room temperature for 3 minutes | | | | |
| NaOBr | | | | |
| 4 drops | 4 drops | 4 drops | 4 drops | 4 drops |

Note the color formed

**4/Lead Acetate Test:**

\*Used for detecting the presence of Sulfur in Cystine.

\*Proteins or peptides containing these amino acid heated with NaOH to split the sulfide group from amino acids.

\*Then, lead acetate react with free sulfide ions resulting in the formation of lead sulfide ( pbs ) a brown to black color.

**Procedure:**

1-Take four separate test tubes. Add 2 ml of: water, 1% egg albumin, 0.02% cystine and 0.02% methionine, respectively.

2-Add 5 ml of 5% NaOH to each test tube.

3-Add few crystals of lead acetate. Incubate all the tubes in boiling water bath for 10 minutes, with occasional mixing of the contents of the tube. Describe the color changes.

|  |  |  |  |
| --- | --- | --- | --- |
| 0.02% methionine | 0.02% cystine | 1% egg albumin | H2O |
| 2 ml | 2 ml | 2 ml | 2 ml |
| 5% NaOH | | | |
| 5 ml | 5 ml | 5 ml | 5 ml |
| Lead Acetate | | | |
| Few crystals | Few crystals | Few crystals | Few crystals |

Incubate in boiling water bath for 10 minutes with occasional mixing.

Describe the color changes in each test tubes.

**5/Folin's Test:**

\*This test for free cystine, therefore protein or peptide must be first hydrolyzed by sodium carbonate and sodium sulfite.

\*Then, Folin's uric acid reagent (phosphotungstic acid) is added which is reduced to tungsten blue.

\*This can be used for the quantitative determination of cystine.

**Procedure:**

1-Take four separate test tubes. Add 1 ml of: water, 0.1% cystine, 1%egg albumin and 0.1% methionine, respectively.

2-Add 7 ml of saturated sodium carbonate to each tube.

3-Add 3 ml of 20% sodium sulfite to each tube.

4-Incubate at room temperature for 5 minutes.

5-Add 1 ml of uric acid reagent to each tube. Note the color formed.

|  |  |  |  |
| --- | --- | --- | --- |
| 0.1% methionine | 1% egg albumin | 0.1% cystine | H2O |
| 1 ml | 1 ml | 1 ml | 1 ml |
| Sodium Carbonate (saturated) | | | |
| 7 ml | 7 ml | 7 ml | 7 ml |
| 20% Sodium Sulfite | | | |
| 3 ml | 3 ml | 3 ml | 3 ml |
| Incubate at R.T for 5 min. | | | |
| Uric acid reagent | | | |
| 1 ml | 1 ml | 1 ml | 1 ml |

Note the color formed