**Lab Sheet 6**

**Detection and quantitative estimation of proteins by biuret method**

**Objectives:**

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**Experiment (1). Qualitative detection of proteins by biuret test:**

**Protocol:**

1. Label three test tubes as **A** and **B**
2. **In tube A:** add 1 ml of animal crude extract.
3. **In tube B:** add 1 ml of water.
4. Add 1 ml of biuret reagent to all tubes and mix well

**Results:**

|  |  |
| --- | --- |
| **Tube** | **Observation** |
| Animal crude extract |  |
| Water |  |

**Experiment (2). Quantitative estimation of proteins by biuret assay:**

**Protocol:**

1. Set up 9 tubes as follows:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tube** | **Water (ml)** | **Bovine serum albumin**  **(BSA) Standard solution**  **(5 g/L)**  **(ml)** | **Sample**  **[unknown concentration] (ml)** | **Biuret reagent** |
| **Blank** | 2 | - | - | 3 ml |
| **A** | 1.6 | 0.4 | - |
| **B** | 1.2 | 0.8 | - |
| **C** | 1 | 1 | - |
| **D** | 0.8 | 1.2 | - |
| **E** | 0.6 | 1.4 | - |
| **F** | 0.4 | 1.6 | - |
| **G** | - | 2 | - |
| **Sample crude extract (D1)** | 1 | - | 1 |
| **Target sample with desire protein (D2)** | 1 | - | 1 |

1. Let the tubes stand at room temperature for **10 min**.
2. Read absorbance at **540 nm** against the blank.
3. Determine the protein contents from BSA standard curve of concentration.

**Results:**

|  |  |  |
| --- | --- | --- |
| **Test tube** | **Protein concentration (g/L)**  **[X- axis]** | **Absorbance at 540 nm [Y- axis]** |
| **Blank** |  |  |
| **A** |  |  |
| **B** |  |  |
| **C** |  |  |
| **D** |  |  |
| **E** |  |  |
| **F** |  |  |
| **G** |  |  |
| **Sample crude extract (D1)** |  |  |
| **Target sample with desire protein (D2)** |  |  |

**Figure**

**Discussion:**

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