**Determination of Protein Concentration**

Sample + DDW 0.5 ml + 1.5 ml of Reagent C

Incubate for 10 minutes at room temperature

Add 150 μl of Folin Phenol reagent (diluted 1:1)

Incubate for 45 minutes at room temperature

Absorbance at 660 nm

**Reagent A:**

|  |  |
| --- | --- |
| Reagent A | 500 ml |
| Na2CO3 | 10 g |
| NaOH | 2 g |
| Sodium tartarate | 0.8 g |
| SDS | 5.0 g |

**Reagent B:**

CuSO4.5H2O 2 g/50 ml

**Reagent C:**

1ml of Reagent B+ 100 ml of Reagent A (Prepare fresh before use).

**Standard for protein determination using BSA in 0.1 M Tris, pH7.4**

Stock : 1 mg/ml BSA in distilled water ( 1000 μg/ml)

Working standard : 0.2 ml of stock A + 1.8 ml of distilled water ( 100 μg/ml)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Volume of standard/stock (μl) | Concentration μg | Volume of DDW ( μl) | Reagent C (ml) | Folin Phenol reagent (μl) | 660 nm |
|  |  |  |  | 1.5 | 150 |  |
|  | **Working standard** |  |  |  |
|  | 100 | 10 | 400 |  |
|  | 200 | 20 | 300 |  |
|  | 400 | 40 | 100 |  |
|  |  |  |  |  |
|  | **Stock** |  |  |  |
|  | 50 | 50 | 450 |  |
|  | 100 | 100 | 400 |  |
|  | 200 | 200 | 300 |  |
|  |  |  |  |  |
|  | **Blank** | - | 400 |  |

*Reference: Anal. Biochem. 87:206-210, 1978.*