Ver.241201

Lowry Protein Assay Kit

PC0030 (100T)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin–Ciocalteu phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids. The final blue colour has a

Kit components

Reagent	Volume	Storage
Reagent A	50mL × 4	RT
Reagent B	5mL × 1	RT
Reagent C	10mL × 1	RT
BSA Standard (5mg/mL)	1mL × 1	-20°C
Reagent D	30mL × 1	RT

BSA standard is stable for 3 months at 4ºC or for 1 year at -20ºC. Other reagents can be kept at room temperature for 1 year.

Reagents and Equipment Required but Not Provided

Centrifuge, micropipette, cooling centrifuge, spectrophotometer / microplate reader, micro glass cuvette / 96-well flat bottom plates and distilled water.

Protocol

Prepare Working Solution

1. Lowry's working solution.
   According to the requirement, mix Reagent A and Reagent B in the ratio 49:1. This mixture is stable for 24 hours at room temperature

2. BSA standard working solution.
   Dilute 10µL BSA standard (5mg/mL) to 100µL with Reagent D to obtain working standard of concentration 0.5mg/mL.

3. Reagent C working solution.
   Dilute 1-part Reagent C with 1-part distilled water before use. Prepare according to the number of samples to be assayed.

Microplate Reader

- Add different volume of BSA standard working solution (0.5mg/mL) to 96 well plates, make up to 20µL with Reagent D as mentioned in the table below.

BSA Working Standard (0.5mg/mL) (µL)	0	2	4	6	8	10	12	14	16	18	20
Reagent D (µL)	20	18	16	14	12	10	8	6	4	2	0
Final Amount of BSA (µg)	0	1	2	3	4	5	6	7	8	9	10

- Add 20µL sample to each well.
- Add 200µL Lowry's working solution to each wells, shake gently and incubate at room

temperature for 10 minutes. Cover the plate properly to avoid evaporation during incubation.

- Add 20µL Reagent C working solution to each well, mix quickly and incubate at 37°C for 30 minutes.
- Measure absorbance at 650nm.
- Draw standard curve with X axis as amount of protein and Y axis as the absorbance.
- Determine sample concentration from standard graph.

Spectrophotometer

- Prepare standards and samples as mentioned in the table below.

	Standard						Sample		
	1	2	3	4	5	6	S1	S2	S3
BSA Working Standard (0.5mg/mL) (µL)	0	40	80	120	160	200	-	-	-
Sample (µL)	-	-	-	-	-	-	200	200	200
Reagent D (µL)	200	160	120	80	40	10	8	6	4
Lowry's working solution (mL)	2	2	2	2	2	2	2	2	2
Mix well and incubate at room temperature for 10 minutes									
Reagent C working solution (µL)	200	200	200	200	200	200	200	200	200
Final Amount of BSA (µg)	0	20	40	60	80	100	-	-	-

- Incubate at 37°C for 30 minutes. Cover the tubes properly to avoid evaporation during incubation.
- Measure absorbance at 650nm.
- Draw standard curve with X axis as amount of protein and Y axis as the absorbance.
- Determine sample concentration from standard graph.

Note:

- If the absorption of the sample is high, dilute appropriately with PBS and repeat the assay.