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Product information

QF 24 TV4
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Lowry Protein Assay Kit

Catalog #: SK4031
Size: 1000 microplate assays or 100 test tube assays
Storage: 4°C (for 1 year)

Product Description:

Lowry's method was the most widely used and cited procedure for protein quantitation. The procedure involves reaction of protein with cupric sulfate and tartrate in an alkaline solution, resulting in formation of tetradentate copper-protein complexes. When the Folin-Ciocalteu Reagent is added, it is effectively reduced in proportion to these chelated copper complexes, producing a water-soluble product whose blue color can be measured at 750 nm. As with other protein assay procedures, the Lowry Protein Assay produces slightly different color response curves for different proteins and can be affected by certain components in the sample buffer. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA), which is included in this kit. A series of dilutions of known concentration are prepared for plotting BSA standard curve, and then the concentration of each unknown is determined based on the standard curve. Quantity range is 5-100µg/ml

Components:

The kit contains Solution A, Solution B, Folin-phenol Reagent, and BSA Protein Standard.
It can carry out 1000 microplate assays or 100 test tube assays.

Components	Volume
Solution A	2 ml
Solution B	100 ml
Folin-phenol reagent	10 ml
BSA protein standard (5mg/ml)	1 ml

Procedures:

A. Microplate assay

1. Plot BSA standard curve.

1.1 Dilute the BSA protein standard to 100 ug/ml.
1.2 Add diluted BSA into 11 wells in microplate as the following:

Solution \ Well #	0	1	2	3	4	5	6	7	8	9	10
DdH2O (µl)	20	18	16	14	12	10	8	6	4	2	0
Diluted BSA (µl)	0	2	4	6	8	10	12	14	16	18	20
BSA final con (µg/ml)	0	10	20	30	40	50	60	70	80	90	100

Procedures (contd.):

1.3 Mix 24µl Solution A and 1.2ml Solution B, transfer 100ul mixture into each well, and then keep at room temperature for 10 minutes.
1.4 Add 10µl Folin-phenol reagent to each well, mix and keep at room temperature for 30minutes.
1.5 Measure A_{750} absorbance value using an Elisa reader.
1.6 Plot protein standard curve.

2. The concentration determination of unknown sample.

2.1 Dilute the unknown sample into appropriate concentration.
2.2 Add 20µl diluted protein sample into two wells, then add 20µl ddH2O into another well as a negative control.
2.3 Mix 6µl Solution A with 0.3ml Solution B, transfer 100µl mixture into each well, then keeps at room temperature for 10 minutes.
2.4 Add 10µl Folin-phenol reagent to each well, mix and keep at room temperature for 30 minutes.
2.5 Measure A_{750} absorbance value using an Elisa reader; calculate the mean value of the two wells of diluted sample.
2.6 Calculate the original concentration of unknown protein sample.

B. Test tube assay

1. Add 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200µl BSA at 100µg/ml into 11 of 1.5ml centrifuge tube. Add appropriate amount of ddH2O into each well to make a final volume of 200µl.
2. Mix 240ul Solution A and 12ml Solution B. Transfer 1ml mixture into each well, mix and keep at room temperature for 10 minutes.
3. Add 100µl Folin-phenol reagent, mix and keep at room temperature for 30 minutes.
4. Measure A_{750} absorbance value using an Elisa reader. Plot BSA standard curve.
5. Add 200µl of diluted protein sample into two wells, and add 200ul ddH2O into another well as a negative control.
6. Mix 40µl of Solution A and 2ml Solution B. Transfer 1ml mixture into each tube. Keep at room temperature for 10 minutes. Add 100µl Folin-phenol reagent, mix and keep at room temperature for 30minutes.
7. Measure A_{750} absorbance value using a spectrophotometer; calculate the mean value of two tubes of diluted sample.
8. Calculate the original concentration of unknown protein sample.

Important Notes:

1. Keep the kit at 4°C, avoid light.
2. The mixture of Solution B and Solution A must be used immediately; residual liquid must be discarded and cannot be used again.
3. Must keep the diluted protein concentration within the BSA standard range.
4. After adding folin reagent, must vortex fully, otherwise the absorbance value will be reduced.
5. Phenol and citric acid will interfere the absorbance.
6. After adding folin reagent, measure the absorbance in 30 minutes, otherwise the absorbance value will be reduced.
7. Only intended for in vitro protein assay.
8. Sample contains a reducing agent or a thiol will turn all tubes (including blank) into dark purple.
9. Sample contains a chelating agent (e.g., EDTA, EGTA) will result in no or decreased color in tubes.