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Product information

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Stable Lowry Protein Assay Kit

Catalog #: SK4051
Size: 1000 preps
Storage: Mixed components storage*

*: Product will be shipped with an icepack. Check storage conditions.

Product Description:

The Lowry procedure involves reaction of protein with cupric sulfate and tartrate in an alkaline solution, resulting in the formation of tetradentate copper-protein complexes. When the Folin-Phenol 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. For the original Lowry method, the alkaline copper-tartrate reagent (Reagent C) must be prepared fresh daily from two other reagents (Reagents A and B). For this assay we have developed a modified cupric sulfate-tartrate reagent that replaces individual Reagents A and B of the original Lowry method with a single stable reagent that substitutes for Reagent C. The color response curves for the Stable Lowry Protein Assay and the original Lowry method have nearly 100% correlation. Accordingly, the Stable Lowry Protein Assay Kit is ideal for loyal Lowry method users who would like the increased convenience of a stable, pre-formulated product. As with other protein assay procedures, the Stable 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. The linear range of this assay is 5-100 µg/ml. This kit is sufficient for 1000 microplate assays or 100 test tube assays.

Features:

- Widely cited in protein research literature.
- Can be used with a standard spectrophotometer or plate reader (750 nm).
- Uses a modified cupric sulfate-tartrate reagent that is stable at room temperature.
- Exhibits good linearity in the range 5 to 100 µg/mL (tested with BSA protein).
- Microplate and cuvette protocols provided.

Composition:

Stable Copper Reagent	100 ml
Folin-Phenol Reagent(1N)	10 ml
BSA Standard (1 mg/ml)	5 ml

Storage and Transportation:

Upon receipt, store Folin-Phenol Reagent(1N) and Stable Copper Reagent at 2-8°C, and protect from light. Store BSA Standard at -20°C. When stored properly, this kit has a one year expiration from time of purchase.

Procedures:

A) Preparation of Diluted BSA (BSA) Standards:

1. Transfer 0, 5, 10, 20, 40, 60, 80, 100 µl of the above BSA standard into separate 2 ml centrifuge tubes, then add the appropriate amount of DDH_2O into each tube so that the total volume is 1000 µl. The final concentration of BSA in each centrifuge tube should be 0, 5, 10, 20, 40, 60, 80, 100µl µg/ml.

B) Test Tube Protein Quantification:

1. Pipette 0.2 ml of each standard and unknown sample replicate into an appropriately labeled test tube.

2. Add 1.0 ml of Stable Copper Reagent to each test tube. Mix well and incubate each tube at room temperature (RT) for exactly 10 minutes.

3. Add 100 µl of Folin-Phenol Reagent(1N) and immediately vortex to mix the contents.

4. Cover and incubate all tubes at RT for 30 minutes.

5. With the spectrophotometer set to 750 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.

6. Subtract the average 750 nm absorbance values of the Blank standard replicates from the 750 nm absorbance values of all other individual standard and unknown sample replicates.

7. Prepare a standard curve by plotting the average Blank-corrected 750 nm value for each BSA standard. Use the standard curve to determine the protein concentration of each unknown sample.

C) Microplate Protein Quantification:

1. Pipette 20 µl of each standard and unknown sample replicate into a microplate.

2. Add 100 µl of Stable Copper Reagent to each well at nearly the same moment using a multi-channel pipettor. Immediately mix microplate on plate mixer for 30 seconds.

3. Cover (e.g., Sealing Tape for 96-Well Plates) and incubate microplate at room temperature (RT) for exactly 10 minutes.

4. Add 10 µl of Folin-Phenol Reagent(1N) to each well using a multi-channel pipettor. Immediately mix microplate on plate mixer for 30 seconds.

5. Cover and incubate microplate at RT for 30 minutes.

6. Measure the absorbance at or near 750 nm on a plate reader.

7. Subtract the average 750 nm absorbance value of the Blank standard replicates from the 750 nm value of all other individual standard and unknown sample replicates.

8. Prepare a standard curve by plotting the average Blank-corrected 750 nm values for each BSA standard. Use the standard curve to determine the protein concentration of each unknown sample.

Notes:

1. Certain substances are known to interfere with the Lowry assay including those with reducing potential, chelating agents, and strong acids or bases. Therefore the use of DTT, ascorbic acid, EGTA, potassium ion, Iron, impure sucrose, tyrosine, uric acid and similar reagents should be avoided as components of the sample buffer.

2. The effects of interfering substances in the Lowry assay may be overcome by removing the interfering substance by dialysis or desalting. Alternatively, the sample may be diluted until the substance no longer interferes, or the proteins may be precipitated with acetone or trichloroacetic acid (TCA) and resuspended in an appropriate buffer.

3. After adding the Folin-Phenol Reagent, absorbance should be read within 60 minutes.