

Product information

QF 24 V4
 V1 Feb 2020

Modified Lowry Protein Assay Kit

Catalog #: SK4041
Size: 1000 preps
Storage: Mixed components storage*

*: Product will be shipped with ice pack. Check storage conditions.
 Products have one year expiration at time of purchase.

Product Description:

Lowry's method is one of the most widely used and cited procedures 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. In the presence of Folin-Phenol Reagent, a water-soluble product is produced in which the blue color be measured at 750 nm. The increase of absorbance at 750 nm is proportional to the amount of protein. However certain substances including those with reducing potential, chelating agents, and strong acids or bases, such as DTT, ascorbic acid, EGTA, Iron, impure sucrose, tyrosine, uric acid and so on, as components of the sample buffer, are known to interfere with the Lowry assay. In this assay kit, a proprietary protein reagent is included that allows removal of most of interference matters to ensure the accuracy of Lowry protein assay. Concentration range for the kit is 5-100 µg/ml.

Features:

- Measure with a standard spectrophotometer or plate reader (750nm).
- Exhibits good linearity in the range 5 to 100 µg/ml.
- Specific protein precipitate reagents can remove the most of interference matters in sample buffer and ensure the accuracy of Lowry protein assay.

Composition:

Solution A	2 ml
Solution B	100 ml
Solution C	50 ml
Folin-Phenol Reagent(1N)	10 ml
BSA Standard (5mg/ml)	1 ml

Storage:

Product shipped at ambient temperature. Upon receipt, store Solution A, B and C at 2-8°C. Store Folin-Phenol Reagent(1N) at 2-8°C and avoid light. Store BSA Standard at -20°C.

Procedures

A) Getting Rid of Interference Matters in Protein Sample Buffer:

1. Add 50 µl Solution C into per 500 µl protein sample solution, mix and keep at 4°C for about 30 minutes. Centrifuge at 15000 rpm for 15 minutes at 4°C to pellet the precipitated protein, decant off the supernatant, remove residual liquid with a pipette, and invert the tube on clean filter paper. Allow the liquid to completely drain from the tube, completely dissolve precipitated protein with DDH₂O.

B) Preparation of Diluted BSA (BSA) Standards:

1. 20 µl of BSA standard into a new centrifuge tube and diluted them with DDH₂O to gain 1000 µl total volume, thus the final concentration of the diluted BSA standard is 100 µg/ml.

2. Transfer 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 μ l of the above diluted BSA standard into each 2 ml centrifuge tube, then add DDH_2O into each 2 ml centrifuge tube, so that the total volume in each centrifuge tube is 20 μ l and the final concentration of BSA in each centrifuge tube is 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μ g/ml.

C) Preparation of Original Lowry Reagents:

1. Mix 50 parts of solution B with one part of solution A (50:1, solution B:A).

NOTE: The original Lowry Reagents must be prepared fresh daily, can store this mixed solution for up to 24 hours at 4°C.

D) Microplate Protein Quantitation:

1. Pipette 20 μ l of each standard or dissolved protein sample into the appropriate microplate wells.
2. Add 100 μ l of the original Lowry 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 μ l 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.

E) Test Tube Protein Quantitation:

1. Pipette 0.2 ml of each standard or dissolved protein sample into appropriately labeled test tubes.
2. Add 1.0 ml of the original Lowry Reagents to each tube, mix well and keep at room temperature for 10 minutes.
3. Add 0.1 ml of the Folin-Phenol Reagent (1N) to each tube, mix well and keep at room temperature for 30 minutes.
4. 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.
5. Subtract the average 750 nm measurements for the Blank replicates from the 750 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 750 nm measurement for each BSA standard, use the standard curve to determine the protein concentration of each unknown sample.

Notes:

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



PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY.
NOT INTENDED FOR HUMAN OR ANIMAL USE.