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BIO BASIC INC.

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BCA Protein Assay Kit

SK3021
QF 24 TV4
CV1 2020

For Research Use Only

BCA Protein Assay Kit
Code: SK3021 (500 Preps)

Features

- Rapid and economical. Entire procedure just takes about 45 minutes (time for preparation of samples is not included).
- Good & widely linear relationship between 20 µg/ml-2000 µg/ml protein ranges.
- High sensitivity.

Description

The BCA Protein Assay kit is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20-2,000 µg/ml). The BCA method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together.

Other Kits Available

- **SK3041: Better Bradford Protein Assay Kit**
  1000 Assays
- **SK3021: BCA Protein Assay Kit (Smith Assays)**
  500 Assays
- **SK3051: Better BCA Protein Assay Kit**
  500 Assays
- **SK4031 Lowry Protein Assay Kit**
  1000 Assays

4. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.

5. Plot standard curve using the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml.

6. Using BSA protein standard curve, calculate concentration of diluted protein sample and original protein concentration with dilution factor X.

The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA. Studies with di-, tri- and tetrapeptides suggest that the extent of color formation caused by more than the mere sum of individual color producing functional groups. Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve. If quantitation of an unknown protein is required, it is advisable to select a protein standard that is similar in quality to the unknown; for example, a bovine gamma globulin (BGG) standard may be used when assaying immunoglobulin samples.

Kit Contents

Components	
BCA Reagent A	100 ml
BCA Reagent B	2 ml
BSA Standard Solution (5 mg/ml)	1 ml

Application

Direct assays of total protein concentration.

Storage

For longer storage, keep all contents cold (4°C).

Protocols for Measurements in Test Tubes

A) Make Dilutions of BSA Standards and Sample of Interest

Label 8 test tubes from #1 to #8. Make BSA serial dilutions as follows:

- In Tube #1, add 100 µl of original BSA protein standard (5 mg/ml) + 900 µl of ddH_2O.
- In Tube #2, transfer 125 µl of ddH_2O + 500 µl from Tube #1.
- In Tube #3, transfer 150 µl of ddH_2O + 450 µl from Tube #2.
- In Tube #4, transfer 150 µl of ddH_2O + 300 µl from Tube #3.
- In Tube #5, transfer 200 µl of ddH_2O + 200 µl from Tube #4.
- In Tube #6, transfer 150 µl of ddH_2O + 150 µl from Tube #5.
- In Tube #7, transfer 100 µl of ddH_2O + 100 µl from Tube #6.
- In Tube #8, transfer 200 µl of ddH_2O ONLY.

This is **BSA standard serial dilution.**

	1	2	3	4	5	6	7	8
BSA final concentration (µg/ml)	500	400	300	200	100	50	25	-

Now dilute sample of interest to preferred concentration (25-500 µg/ml). Record dilution factor X.

Label Tubes #9 to #11

- In Tube #9, transfer 100 µl of diluted protein sample of interest.
- In Tube #10, transfer 100 µl of diluted protein sample of interest, preferred at a different dilution factor.
- In Tube #11, transfer 100 µl of ddH_2O ONLY.

NOTE: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

B) Measure Concentration of BSA Standards and Sample of Interest

1. Transfer 25 µl of BSA serial dilutions (step A) from each tube #1 to #8 to each well in a 96-well plate. Make duplicates as a good laboratory practice. Add 200 µl of Working Reagent to each tube. Mix gently. Cover and incubate the plate at selected temperature and time:

   - Standard Protocol: 37°C for 30 minutes.
   - RT Protocol: RT for 2 hours.
   - Enhanced Protocol: 60°C for 30 minutes.

   NOTE: Increasing the incubation time or temperature increases the net 562 nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of the protocol.

   NOTE: Use heated oven to heat samples for either Standard (37°C incubation) or Enhanced (60°C incubation) Protocol. Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.

2. Cool all tubes to room temperature.

3. Measure the absorbance at 562 nm on a plate reader.

This is **BSA standard serial dilution.**

	1	2	3	4	5	6	7	8
BSA final concentration (µg/ml)	500	400	300	200	100	50	25	-

Label Tubes #9 to #11

- In Tube #9, transfer 20 µl of diluted protein sample of interest.
- In Tube #10, transfer 20 µl of diluted protein sample of interest, preferred at a different dilution factor.
- In Tube #11, transfer 20 µl of ddH_2O ONLY.

Preparation of the BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

(# Standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required

Example: for 3 unknowns and 2 replicates of each sample:
(8 standards + 3 unknowns) × (2 replicates) × (200 µl) = 4.4 ml WR required

2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A: B). For the above example, combine 50 ml of Reagent A with 1 ml of Reagent B.

B) Preparation of the BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

(# Standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required

Example: for 3 unknowns and 2 replicates of each sample:
(8 standards + 3 unknowns) × (2 replicates) × (1 ml) = 22 ml WR required

2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A: B). For the above example, combine 50 ml of Reagent A with 1 ml of Reagent B.

NOTE: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

C) Measure Concentration of BSA Standards and Sample of Interest

1. Transfer 100 µl of BSA serial dilutions (step A) from each tube #1 to #8 to a new clean tube. Make duplicates as a good laboratory practice. Add 1 ml of Working Reagent to each tube. Cover and incubate tubes at selected temperature and time:

- Standard Protocol: 37°C for 30 minutes.
- RT Protocol: RT for 2 hours.
- Enhanced Protocol: 60°C for 30 minutes.

NOTE: Increasing the incubation time or temperature increases the net 562 nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of the protocol.

NOTE: Use a water bath to heat tubes for either Standard (37°C incubation) or Enhanced (60°C incubation) Protocol. Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.

2. Cool all tubes to RT.

3. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.

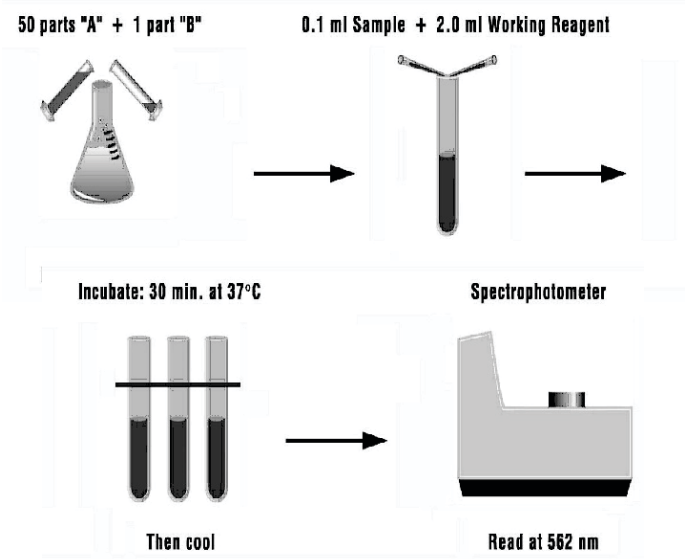
   NOTE: Because the BCA assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of each other.

4. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.

5. Plot standard curve using the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml.

6. Using BSA protein standard curve, calculate concentration of diluted protein sample and original protein concentration with dilution factor X.

Procedure Summary



Protocols for Measurements in 96-Well Plates

A) Make Dilutions of BSA standards and Sample of Interest

Label 8 micro-centrifuge tubes from #1 to #8. Make BSA serial dilutions as follows:

- In Tube #1, add 30 µl of original BSA protein standard (5 mg/ml) + 270 µl of ddH_2O.
- In Tube #2, transfer 40 µl of ddH_2O + 160 µl from Tube #1.
- In Tube #3, transfer 40 µl of ddH_2O + 120 µl from Tube #2.
- In Tube #4, transfer 50 µl of ddH_2O + 100 µl from Tube #3.
- In Tube #5, transfer 50 µl of ddH_2O + 50 µl from Tube #4.
- In Tube #6, transfer 50 µl of ddH_2O + 50 µl from Tube #5.
- In Tube #7, transfer 50 µl of ddH_2O + 50 µl from Tube #6.
- In Tube #8, transfer 50 µl of ddH_2O ONLY.