T-Pro BCA Protein Assay Kit

Store at RT

(JB04-D001) 500 assay
reagent A 500ml
reagent B 12ml
Albumin standard (2mg/ml) 5*1ml (store at 2~8℃)

T-Pro Biotechnology

This product is for laboratory research ONLY and not for diagnostic use.

Description	T-Pro 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 was base on the reduction of Cu+2 to Cu+1 by protein in an alkaline medium with the highly sensitive and selective colorimetric detection. The purple-colored reaction product of this assay is formed and 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 reaction occurred in T-Pro BCA Protein Assay Kit 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. Thus, protein concentrations generally are determined and reported with reference to standards of 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.
Storage	T-Pro BCA Protein Assay Kit is stable for RT

Procedural

Preparation of Standards and Working Reagent

Preparation of Diluted Albumin (BSA) Standards.

1. **Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as the sample(s). Each 1 ml ampule of 2.0 mg/ml Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard.**

Preparation of the T-Pro Protein Assay Working Reagent (WR).

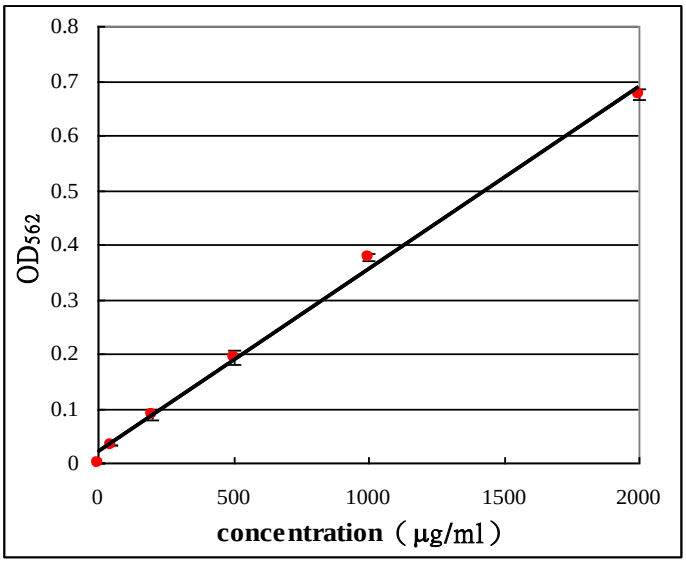
1. **Use the following formula to determine the total volume of WR required: (# standards + # unknowns) x (# replicates) x (volume of WR per sample) = total volume WR required.**
2. **Prepare WR by mixing 50 parts of T-Pro BCA Protein Assay Reagent A with 1 part of T-Pro BCA Protein Assay Reagent B (50:1, Reagent A:B).**

Procedure Summary

a. Test Tube Procedure

1	Pipette 0.1 ml of each standard and unknown sample replicate into an appropriately labeled test tube.
2	Add 1.0 ml of the WR to each tube and mix well.
3	Cover and incubate tubes at selected temperature and time: ● Standard Protocol: 37°C for 30 minutes (working range = 20-2,000 µg/ml) ● RT Protocol: RT for 2 hours (working range = 20-2,000 µg/ml) ● Enhanced Protocol: 60°C for 30 minutes (working range = 5-250 µg/ml) Notes: 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.
4	Cool all tubes to RT.
5	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. Notes: Because the T-Pro Protein 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.
6	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.
7	Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

Figure 1: Typical color response curves for BSA using the Standard Test Tube Protocol



b. Microplate Procedure (Sample to WR ratio = 1:8)

1. Pipette 25 µl of each standard or unknown sample replicate into a microplate well (working range = 20-2,000 µg/ml).
2. Add 200 µl of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate at 37°C for 30 minutes.
4. Cool all tubes to RT.
5. Measure the absorbance at or near 562 nm on a plate reader.

   Notes:
   - Wavelengths from 540-590 nm have been used successfully with this method.
   - Because plate readers use a shorter light path length than cuvette spectrophotometers, the Microplate Procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard Test Tube Procedure. If higher 562 nm measurements are desired, increase the incubation time to 2 hours.
   - Increasing the incubation time or ratio of sample volume to WR increases the net 562 nm measurement for each well and lowers both the minimum detection level of the reagent and the working range of the assay. As long as all standards and unknowns are treated identically, such modifications may be useful.
6. 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.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

Table1、Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20–2,000 µg /ml)			
Vial	**Volume of Diluent**	**Volume and Source of BSA**	**Final BSA Concentration**
A	0	300 µl	2000 µg /ml
B	125 µl	375 µl	1500 µg /ml
C	325 µl	325 µl	1000 µg /ml
D	175 µl	175 µl of vial B dilution	750 µg /ml
E	325 µl	325 µl of vial C dilution	500 µg /ml
F	325 µl	325 µl of vial E dilution	250 µg /ml
G	325 µl	325 µl of vial F dilution	125 µg /ml
H	400 µl	100 µl of vial G dilution	25 µg /ml
I	400 µl	0 l	0 µg /ml
Dilution Scheme for Enhanced Test Tube Protocol (Working Range = 5–250 µg /ml)			
Vial	**Volume of Diluent**	**Volume and Source of BSA**	**Final BSA Concentration**
A	700 µl	100 µl	250 µg /ml
B	400 µl	400 µl of vial A dilution	125 µg /ml
C	450 µl	300 µl of vial B dilution	50 µg /ml
D	400 µl	400 µl of vial C dilution	25 µg /ml
E	400 µl	100 µl of vial D dilution	5 µg /ml
F	400 µl	0 l	0 µg /ml