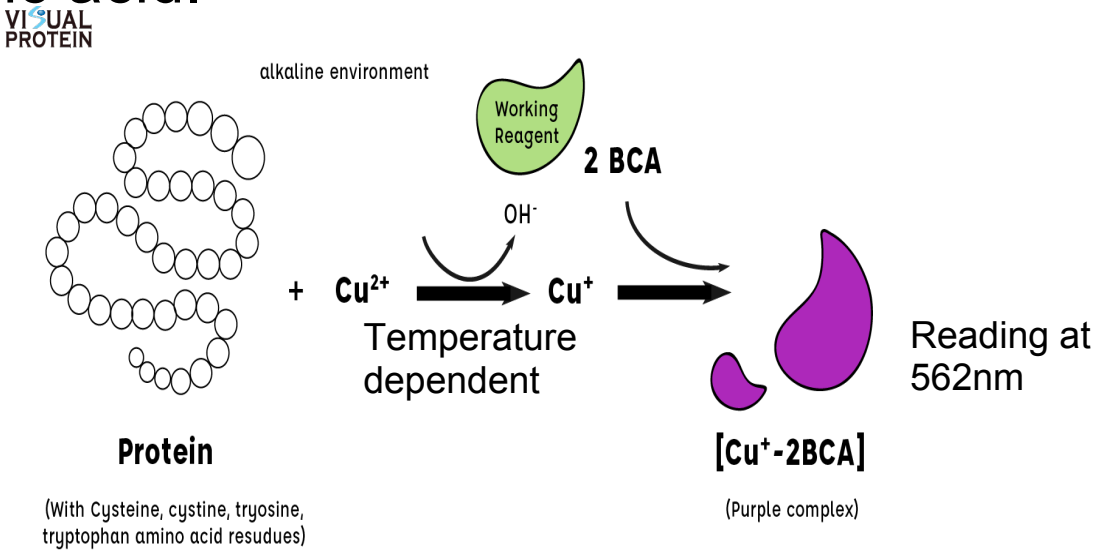
BCA protein assay kit

Objective

- Use Bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein.

Background

- Method combines the reduction of Cu^{+2} (Cupric ion) to Cu^{+1} (Cuprous ion) by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid.



- Water-soluble complex exhibits a strong absorbance at 562nm that is nearly linear with increasing protein concentrations over a broad working range (20-2000µg/mL).

Procedure: Create standard curve

- Create a standard curve of known concentrations of Bovine serum albumin

Preparation of Standards and Working Reagent (required for both assay procedures)

A. Preparation of Diluted Albumin (BSA) Standards

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 1mL ampule of 2mg/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.

Table 1. Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20-2,000µg/mL)

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

Preparation of working reagent

Everybody - Make 5 ml of WR
5 ml Reagent A + 100 µl Reagent B
Assay in 96-well plates
200µl WR for 22 total samples
(9 standards in duplicate + 2 unknowns
In duplicate)

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 the standard test-tube procedure with 3 unknowns and 2 replicates of each sample:

   (9 standards + 3 unknowns) × (2 replicates) × (2mL) = 48mL WR required

   Note: 2.0mL of the WR is required for each sample in the test-tube procedure, while only 200 µl of WR reagent is required for each sample in the microplate procedure.

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 50mL of Reagent A with 1mL 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).

Microplate procedure part 1

1. Pipette 25µL of each standard or unknown sample replicate into a microplate well (working range = 20-2000µg/mL) (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041).

2. Add 200µL of the Working reagent (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 plate to RT.

5. Measure the absorbance at or near 562nm on a plate reader.

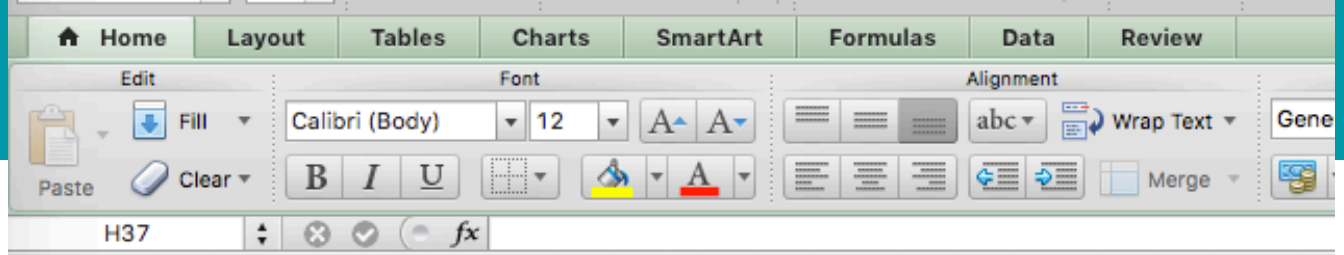
Microplate procedure part 2

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

7. Prepare a standard curve by plotting the average Blank-corrected 562nm measurement for each BSA standard vs. its concentration in µg/mL – use excel.

8. Use the standard curve to determine the protein concentration of each unknown sample.

9. See demo/instructions in next slide



	A	B	C	D	E	F
1	BCA protein kit					
2						
3	STEP1 - Build the following table X axis is concentration (µg/ml) and Y axis is Abs 562 nm					
4	Standard concentration (µg/ml)	Abs 562nm replicate	Abs 562nm replicate	AVERGE	AVERAGE - BLANK	
5	0					
6	25					
7	125					
8	250					
9	500					
10	750					
11	1000					
12	1500					
13	2000					
14	Unknown1					
15	Unknown2					
16						
17	STEP2 - Copy and past the standards only and the corresponding AVERAGE -BLANK - do not include 0					
18	Standard concentration (µg/ml)	Abs 562 nm (FINAL: AVERAGE - BLANK)				
19	25					
20	125					
21	250					
22	500					
23	750					
24	1000					
25	1500					
26	2000					
27						
28						
29	STEP3 - Create a chart: scattered plot					
30	Add title, X axis label with units and Y axis label with units					
31	Select your chart					
32	click on add trendline - linear					
33	click on the trendline					
34	select format trendline					
35	chose display trendline equation and R^2 value					
36						
37	STEP4 - Solve the equation to determine the protein concentration of unknowns # 1 and #2					