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Better BCA Protein Assay Kit

SK3051
QF 24 TV4
CV1 2020

For Research Use Only

Better BCA Protein Assay Kit

Code: SK3051 (500 Preps)

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Description

The Better BCA Assay Kit can be used for quantitation of total protein in samples while minimizing interference from disulfide reducing agents. The BCA assay is based on the well-known reduction of Cu^{2+} to Cu^+ by protein in an alkaline medium and the highly sensitive and selective colorimetric detection of the cuprous cation using bicinchoninic acid (BCA). Disulfide reducing agents, particularly dithiothreitol, 2-mercaptoethanol and TCEP are also capable of reducing Cu^{2+} to Cu^+ . To minimize the affect of these copper reducers, a compatibility reagent that modifies disulfide reducing agents is added to the sample before adding the BCA reagents. This assay is also compatible with most ionic and non-ionic detergents in the presence of a disulfide reducing agent. Purification of membrane proteins presents unusual challenges to protein quantitation, as these proteins often require the presence of detergents and a disulfide reducing agent to maintain solubility and stability. The dual compatibility of this kit enables researchers to more accurately determine protein concentration for such samples. The kit is compatible with protein samples containing up to 5 mM DTT, 10 mM 2-mercaptoethanol or 10 mM TCEP.

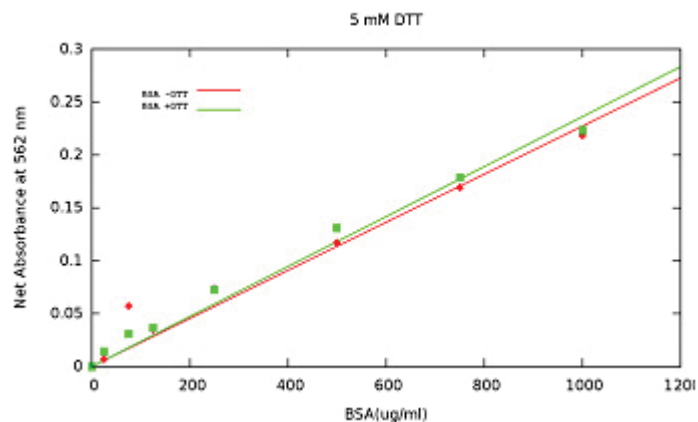
9. The following are lists of substances compatible with BCA assays.

Substance	Compatible Concentration
NP-40	5.00%
Emulgen	1.00%
HEPES	100 mM
DTT	5 mM
Guanidine HCl	4.0 M
Triton X-100	5.00%
Octyl- β -Glucoside	5.00%
Urea	3.0 M
Sucrose (fresh)	40%
Glycine, pH 2.8	100mM
Glucose (fresh)	10mM
EDTA	10mM
Sodium Chloride	1.0M
NaOH	0.1M
Ammonium Sulfate	1.5M
Sodium Acetate, pH 5.5	200mM
SDS	5.00%
Brij-35	5.00%
Lubrol	1.00%
CHAPS	5.00%



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NOT INTENDED FOR HUMAN OR ANIMAL USE

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



Notices

- The kit has a one year expiration.
- The linear curve range of quantitation sample should be between 25-1000 $\mu\text{g/ml}$. If the sample concentration exceeds the range, please dilute the sample first.
- The working stock solutions must be prepared fresh; Any residual amount must be discarded and can not be for further use.
- Must measure the absorbance value in 10 minutes.
- If sample contains lipid, lipoprotein and/or biogenic amines, the absorbance value will increase. If sample contains Cu ion, the absorbance value will decrease. Please use DOC-TCA methods to remove them.
- Solution A and solution B mixture may become turbid. But after vortexing, solution should become clear.
- After incubate at 37°C for 30 minutes, slowly cool down to room temperature before measuring.
- The kit only can be used for in vitro experiment.

Kit Contents

The kit contains Solution A, Solution B, Solution C, Solution D and BSA protein standard. The kit can be used for up to 500 assays.

Components	
Solution A*	100 ml
Solution B	10 ml
Reagent C	260 mg
Solution D	50 ml
BSA protein standard (2 mg/ml)	10 x 1 ml

*Solution A may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37°C.

Storage

The kit must be kept at 4°C and away from direct light.

Protein Assay Selection Guide

Important Substance Compatibilities

Assay Product	Detention (Amax)	Compatible	Incompatible	Uniformity
BCA	562 nm	Detergents	Reducing Agents; Chelators	High
Better BCA	562 nm	Detergents; Reducing Agents	Chelators	High
Bradford	595 nm	Most Reducing Agents; Chelators	Detergents	Low

Procedures

A) Make Dilutions of BSA standards and Sample of Interest

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

- In Tube #1, add 120 μ l of original BSA protein standard (2 mg/ml) + 120 μ l of Solution D.
- In Tube #2, transfer 50 μ l of Solution D + 150 μ l from Tube #1.
- In Tube #3, transfer 50 μ l of Solution D + 100 μ l from Tube #2.
- In Tube #4, transfer 80 μ l of Solution D + 80 μ l from Tube #3.
- In Tube #5, transfer 80 μ l of Solution D + 80 μ l from Tube #4.
- In Tube #6, transfer 40 μ l of Solution D + 60 μ l from Tube #5.
- In Tube #7, transfer 40 μ l of Solution D + 20 μ l from Tube #6.
- In Tube #8, transfer 80 μ l of Solution D ONLY.

This is **BSA standard serial dilution**.

	1	2	3	4	5	6	7	8
BSA final concentration (μ g/ml)	1000	750	500	250	125	75	25	-

Now dilute sample of interest to preferred concentration (125-500 μ g/ml). Record dilution factor X.

Label Tubes #9 to #11.

- In Tube #9, transfer 25 μ l of diluted protein sample of interest.
- In Tube #10, transfer 25 μ l of diluted protein sample of interest, preferred at a different dilution factor.
- In Tube #11, transfer 25 μ l of Solution D ONLY.

B) Make 1X Working Stock Solution A

Add 4 Volume of ddH₂O with 1 volume of Solution A to make 1X Solution A.

C) Make Working Stock - AB

Mix 10 ml of 1X solution A and 200 μ l solution B into a new 10 ml centrifuge tube. This is working stock-AB.

D) Make Working Stock - CD

Mix 250 μ l ddH₂O with 250 μ l solution D, vortex. Then transfer 10 mg reagent C into the above centrifuge tube, vortex for 1 minute. This is working stock - CD.

1. Transfer 25 μ l 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 25 μ l of working stock solution-CD. Vortex and keep at 37°C for 30 minutes.

NOTE: For microplate assays, transfer 5 μ l of BSA serial dilutions from each tube #1 to #8 into wells of a 96-well plate. Make duplicates as a good laboratory practice. Add 5 μ l of working stock solution-CD. Vortex and keep at 37°C for 30 minutes.

2. Transfer 1 ml of working stock-AB into each tube from step Vortex and keep at 37°C for 30 minutes. Slowly cool down to room temperature.

NOTE: For microplate assays, transfer 200 μ l of working stock- AB into each tube well. Mix or vortex and keep at 37 °C for 30 minutes. Slowly cool down to room temperature.

3. Measure absorbance A562 using a spectrophotometer.
4. Repeat step 3 to 5. Calculate mean absorbance A562 values and standard deviations.
5. Plot standard curve.