

BCA Assay

Aims of the experiment

BCA assay is designed for determining protein content in our homemade cell extract. The procedure is according to the #7780 Kit from Cell Signaling Technology, Inc. Buffer is prepared according to Sun, Z. Z et al., 2013.

Materials

- Commercial BCA Assay Kit
- S30B buffer with and without DTT
- Cell extract produced from Dialysis Protocol
- 1.5 micro-centrifuge tubes
- 200µl micro-centrifuge tubes
- 15/50ml Falcon Tube
- Stepper pipette
- Multipipette
- 96-well microplate
- Plate reader

Preparation

Always keep the cell extract on ice even after mixing with the solution!

1. Dilute the contents of one Albumin Standard (BSA) ampule provided from the Kit into several micro-centrifuge tubes according to the table below.

Use the S30B buffer without DTT for dilution.

Vial	Diluent/Sample Buffer Volume (µl)	BSA Source and Volume (µl)	Concentration (µg/ml)
A	0	200 of stock	2,000
B	66	200 of stock	1,500
C	100	100 of vial A	1,000
D	100	100 of vial B	750
E	100	100 of vial C	500
F	100	100 of vial E	250
G	100	100 of vial F	125

2. Store the excess undiluted BSA standard (2 mg/ml) in a sterile micro-centrifuge tube at 4°C for up to 2 weeks.

Procedure

1. Preheat plate reader at 37°C.
2. Dilute the extract sample in S30B buffer for triplicate measurement, with the final protein concentration at around 1mg/ml.
3. Preapre Working Reconstitution Buffer: dilute the Reconstitution Buffer 1:1 with ddH₂O.
4. Prepare Compatibility Reagent Solution: puncture the foil covering on the Compatibility Reagent tube with a clean pipette tip. Each sample requires 4µl Compatibility Reagen Solution.
5. Add 100µl of Working Reconstitution Buffer into the tube and dissolve by stirring.
6. Keep it on ice and protect from light.
7. Prepare BCA Working Reagent (WR): calulate the required total volume of WR by using the formula below:

$(\# \text{ controls} + \# \text{ standards} + \# \text{ unknowns}) \times (\# \text{ replicates}) \times (\text{volume of WR per sample}) = \text{total volume WR required.}$
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- I. Example: For three unknowns and two replicates of each sample:
 - II. $(2 \text{ controls} + 7 \text{ standards} + 3 \text{ unknowns}) \times (2 \text{ replicates}) \times (0.26\text{ml}) = 6.24\text{ml WR required.}$
 - III. Mix 50 parts BCA Reagent A with 1 part of BCA Reagent B (total: 51 parts).
 - IV. Example: $6.24\text{ml WR} = 6.13\text{ml Reagent A} + 0.13\text{ml Reagent B}$
8. Preapre Assay:
 - I. Add 4µl Compatibility Reagent Solution using stepper pipette into required well on a microplate.
 - II. Add 9µl of BSA Standard (duplicate), Standard Control (buffer w/o DTT) (triplicate) and Sample Control (buffer w/ DTT) (triplicate) or extract sample (triplicate) to individual well.
 - III. Add 260µl WR to each well using multipipette.
 - IV. Cover the plate and spin in a plate shaker for 1 minute.

- V. Use the Standard Control as the blank. Shake and incubate in plate reader at 37°C for 30 mins, then measure the absorbance of the samples at 562nm.