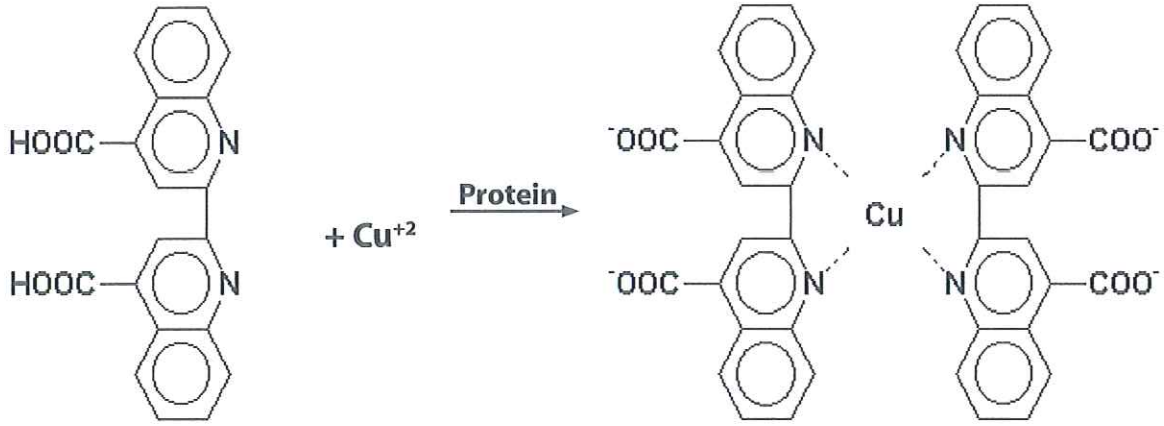
Short Manual

BCA-Protein Quantification Assay for the Carbonyl Protein ELISA K 7822BCA

Principle of the BCA protein quantification method

This protein quantification kit contains bicinchoninic acid (BCA) disodium salt and copper-II-ions. Proteins are able to reduce bivalent copper ions to monovalent copper ions (Smith et al., 1985). The monovalent copper ions obtained by this reaction form a specific color complex with the bicinchoninic acid (BCA) in the test solution. The absorption of the color complex is measured by a spectral photometer. The intensity of the color correlates with the protein concentration.



The BCA protein quantification assay is characterized by a high sensitivity and low background interference.

Content of the Kit sufficient for 96 determinations in a microtiter plate

- Microtiter plate (PLATE)
- Solution A (SOL A): 24 ml
- Solution B (SOL B): 0.5 ml
- Standard stock solution (STD): 0.5 ml
- Adhesive cover foil

Material required but not supplied

- Bidistilled water (aqua bidist.)
- Precision pipettors and disposable tips to deliver 20 - 1000 µl
- Incubator at 37°C
- Standard laboratory glass or disposable plastic vials
- Microtiter plate reader at 540 - 590 nm

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Preparation

Preparation of the reaction mixture

Mix SOL B (Solution B) with 50-fold volume of SOL A (Solution A).

For one plate, e.g.: 21 ml SOL A + 420 µl SOL B
For 24 wells, e.g.: 5.5 ml SOL A + 110 µl SOL B

To run assay more than once, ensure that reagents are stored at conditions stated on the label. Prepare only the appropriate amount necessary for each assay. The kit can be used up to 4 times within the expiry date stated on the label.

Preparation of standard curve dilution series

Prepare standard dilution series fresh before each assay from the STD (standard stock solution, 500 µg/ml).

Standard 1: 100 µl Standard stock solutin + 100 µl aqua bidist. (250 µg/ml)
Standard 2: 100 µl Standard 1 + 100 µl aqua bidist. (125 µg/ml)
Standard 3: 100 µl Standard 2 + 100 µl aqua bidist. (62.5 µg/ml)
Standard 4: 100 µl Standard 3 + 100 µl aqua bidist. (31.25 µg/ml)
Standard 5: 100 µl Standard 4 + 100 µl aqua bidist. (15.6 µg/ml)
Standard 6 (corresponds to **blank**): 100 µl aqua bidist. (0 µg/ml)

Assay Procedure

- Pipet 25 µl of each standard, blank or sample into the PLATE (microtiter plate) wells in duplicates
- Add 200 µl per well of the freshly prepared reaction mixture
- Cover the PLATE with a foil
- Incubate for 3 h at 37 °C
- Determine the absorption at 540 - 590 nm

Evaluation of the results

A dose response standard curve of the absorbance unit (optical density, OD at 590 nm) vs. concentration is generated using the values obtained from the standard. The use of a linear regression is recommended. The concentration of the samples is determined directly from the linear standard curve.

References

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