PanReac AppliChem
ITW Reagents

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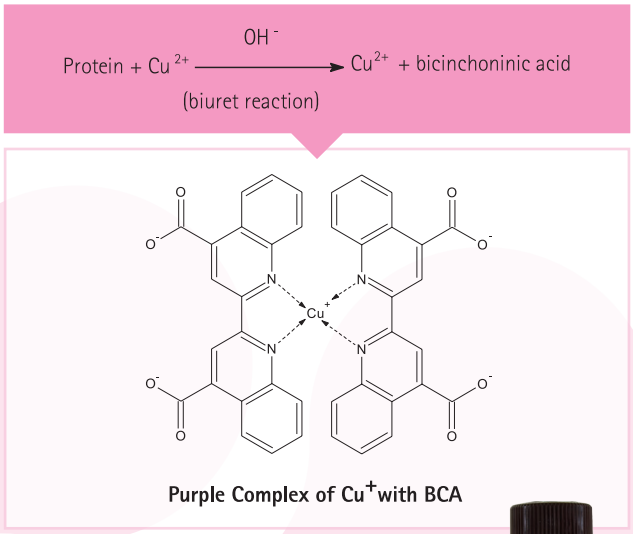
# Bicinchoninic Acid (BCA) Protein Assay

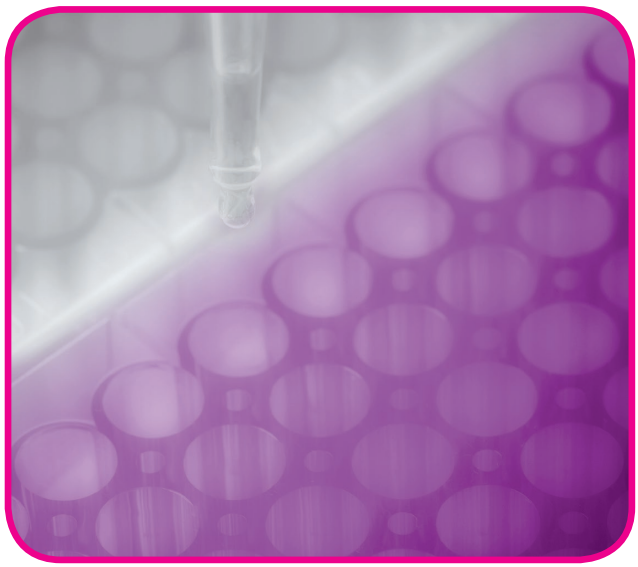
**Bicinchoninic acid Protein Assay** kit is a formulation based on bicinchoninic acid for the rapid and sensitive detection and quantitation of total protein content. The Bicinchoninic acid method is faster and easier than Lowry, with much greater tolerance to interference from non-ionic detergents and buffer salts.

The Bicinchoninic acid method combines the biuret reaction, i.e., the reduction of $Cu^{2+}$ ions to $Cu^{+}$ by proteins in an alkaline medium with complexation of the latter with bicinchoninic acid. The purple-colored Cu(bicinchoninic acid)$_2$ $^{3-}$ complex displays a strong absorbance at 562 nm which is proportional to protein concentration over a broad working range of 20 - 2000 µg/ml.






Purple Complex of $Cu^{+}$ with BCA



## Main Advantages

- Photometric method (reading at 562 nm).
- More sensitive than Lowry and less subject to interferences. In particular, it is insensitive to detergents such as Triton® X-100 and SDS (1 %).
- Linear working range for BSA is 20 - 2000 µg/ml for the Bicinchoninic acid Protein Assay.
- Less protein-to-protein variation than Coomassie and Bradford-based assays.
- Reagents are stable two years at room temperature.
- Adaptable to microplate format.

## Ordering Information

| Description | Code | Packaging |
|---|---|---|
| **Bicinchoninic Acid Protein Assay** | A7787,0500 | 500 Tests |


### Kit components

- Reagent A: BCA/tartrate in alkaline carbonate buffer (1 L)
- Reagent B: 4 % $CuSO_4 \cdot 5H_2O$ (25 ml)

**Storage:** RT (Room Temperature)

### Stability

- Reagents A + B: 2 years
- Working reagent: several days

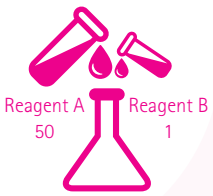
**Sensitivity:** 20 - 2000 µg/ml protein

**No. of tests:** 500 test tubes or 5000 microplate assays

# PROCEDURE

## Preparation of the Working Reagent

Mix 50 parts of Reagent A and 1 part of Reagent B. The amount of working reagent required for each sample is 2.0 ml for the Test Tube Procedure and 200 µl for the Microplate Procedure.

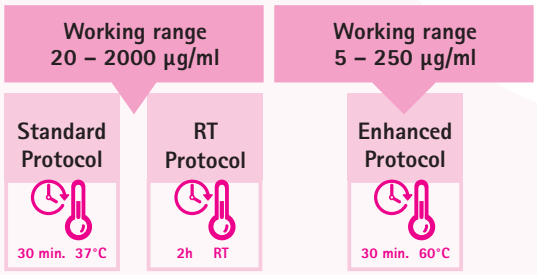



## Preparation of the Calibration Standards

Prepare a fresh set of protein standards in the 20-2000 µg/ml range, preferably using the same diluent as your sample. The standards are generally prepared from a 1.0-2.0 mg/ml stock solution of Bovine Serum Albumin (code A1391). For several proteins a Correction Factor relative to BSA is reported in the table included in the protocol.

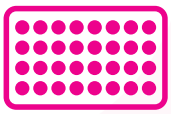
## Test tube Procedure

1. Pipette 0.1 ml of each standard (including a blank) and unknown sample into a labeled test tube.
2. Add 2.0 ml of working reagent to each tube and mix thoroughly.
3. Close / Cover the tubes and incubate at a selected temperature and time in a water bath.




4. Cool the tubes to RT.
5. Measure the absorbance at 562 nm of all the samples within 10 minutes.
6. Subtract the 562 nm absorbance value of the blank from the readings of the standards and the unknowns.
7. Plot the blank-corrected 562 nm reading for each standard vs. its concentration. Determine the protein concentration of each unknown from the calibration plot.

## Microplate procedure



1. Pipette a 25 µl of each standard (including a blank) and unknown sample into a microwell plate.
2. Add 200 µl of working reagent to each well and mix thoroughly.
3. Cover the plate and incubate at **37°C for 30 min.** Do not incubate at higher temperatures.

4. Cool plate to RT.
5. Measure the absorbance at 562 nm of all the samples on a plate reader.
6. Subtract the 562 nm absorbance value of the blank from the readings of the standards and the unknowns.
7. Plot the blank-corrected 562 nm reading for each standard vs. its concentration. Determine the protein concentration of each unknown from the calibration plot.

**Note:**

The test tube procedure requires a larger volume (0.1 ml) of protein sample but, since the sample to working reagent ratio is 1/20, the effect of interfering substances is minimized.

The microplate procedure requires a smaller volume (10-25 µl) of protein sample but, since the sample to working reagent ratio is 1/8, it offers less flexibility in overcoming the effect of interfering substances and obtaining a low level of detection.



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