D22. Protein Concentration Determination Using BCA Procedure (96-Well Plate)

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REAGENTS:

PIERCE/Fisher Scientific: BCA Protein assay kit (**Catalog# 23226** or similar products).

BSA Standard Stock: 2ug/ul (**Note**: you can use the **NEB**'s BSA control for restriction enzymes).

BCA Protein Assay Procedure（96-well plate format）

1) Mix 50 parts BCA **Reagent A** with 1 part **Reagent B** (**Note**: you will need 50ul per well/assay).
2) Add 50ul to each well of 96-well plates.
3) Add 5ul samples (e.g., from 100ul lysate of one well from 12- or 24-well plates) or BSA Standard (0ug, 1ug, 2ug, 3ug, 4ug, 8ug BSA) to each well.
4) Mix and incubate for 15 minutes @ 60ºC (**Note**: Incubation Temp and/or Time may vary, e.g., 37°C to 60°C and 15min to 30min, depending on protein concentrations. **We recommend using 15min x 60°C**).
5) Add 50ul ddH_2O to each well and mix.
6) Cool down to RT.
7) Read OD at 562nm using microplate reader.

Note: The **Cell Lysis Buffer (5x stock, from Promega/Fisher, Cat# PR-E1531)** yields a purple color. Thus, the **blank control** should accompany each batch of assays (i.e., add 5ul 1x Cell Lysis Buffer in 50ul BCA A/B mixture; and incubate/read at the same condition as for the samples).