BCA Assay

Technic: BCA Assay

Goal: Measure the total amount of proteins contained in the exosome samples

Procedure:

A. Preparation of standards and working solutions

Dilute the BSA standard into several clean vials, preferably using the same diluent as the samples.

Use the following table as a guide to prepare a set of BSA standards. Each 100 µL of BSA standard is sufficient to prepare a set of diluted standards for either working range suggested in the table.

Table 1 BSA standard dilution scheme for the microplate procedure (working range = 5 - 250 µg/mL)

Vial	Volume of diluent	Volume and source of BSA	Final BSA concentration
A	87.5 µL	12.5 µL of stock	250 µg/mL
B	50 µL	50 µL of vial A solution	125 µg/mL
C	60 µL	40 µL of vial B solution	50 µg/mL
D	50 µL	50 µL of vial C solution	25 µg/mL
E	50 µL	20 µL of vial D solution	5 µg/mL
F	100 µL	0	0 = blank

Perform a 1/10 and 1/100 dilution of the recovered exosomes, which have been analyzed by NTA, in filtered 1X PBS.

B. Prepare BCA working reagent (WR)

Prepare WR by mixing 50 parts of BCA reagent A with 1 part of BCA reagent B (50:1, Reagent A:B). For the above example, combine 50 mL of reagent A with 1mL of reagent B.

C. Microplate procedure

1. Pipette 25 µL of each standard or unknown sample replicate into a 96-well plate
2. Add 200 µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover the plate and incubate at 37°C for 30 minutes.

4. Equilibrate the plate to room temperature. Measure the absorbance at or near 562 nm on a plate reader.

Reagents and Solutions:

- BSA solution stock (2000 µg/mL)
- Pierce™ BCA Protein Assay Kits

Equipment:

- 96-well plate
- plate skaer
- 37°C incubator
- microplate reader at 562 nm
- microtubes

Estimate time:
~1h