BCA assay

Protocol for estimating the protein content of EVs

Use the buffer your EVs in as reagent blank control from the first step!

1. Add 2,5 µl cell lytic buffer to 5 µl EV sample (and the reagent blank) in a PCR tube and add 17,5 µl water
2. Freeze and thaw it at -20°C 3 times (about 5 minutes in enough for freezing)
3. Pipette 50 µl from the premade standards into labelled Eppendorf tubes
   Standards: A,B,C,D,E,F,G, Ø Blank (8 in total)
   [µg/ml]: 0, 2, 5, 10, 25, 50, 100, 200
4. Prepare and add the BCA working solution:
   in each sample (reagent blank) and standard, put equal volume of working solution A:B:C reagents in 25:24:1 ratio (50 µl for the standards and 25 µl)

   Note: calculate the needed working solution for about 1-2 more samples than you actually have, so that you can be sure to have enough!

   E.g.: if you have 6 samples and the reagent blank and you also need the 8 standards, so all together need 7x25 + 8x50 = 575 µl of working solution.
   The A:B:C ratio is 25:24:1, so you need (575/50)*25 = 287,5 µl from A, 274 µl from B and 11,5 µl from C.

5. Incubate for about 1 hour at 60°C (if you have much protein, than less time is enough)
6. Measure in NanoDrop