Quantification of proteins

Solutions & Materials

Loading buffer: 1% SDS, 10 mM tris, 10% glycerol in Milli-Q-water

Protein of comparison: HA-tagged protein (for quantification on the western blot)

Assay: Pierce™ BCA Protein Assay Kit (Thermo Scientific, Product number #23225)

Other materials: 96 well plate

Freeze-drying / lyophilizer

1. Transfer 2 ml of your culture into a 2 ml reaction tube.
2. Centrifuge at 5.000 g for 5 minutes. Transfer supernatant into a fresh tube.
3. Repeat step 2, transfer the supernatant into a 15 ml centrifuge tube.
4. Cut transparent tights (nylon tights) into 4 x 4 cm squares.
5. To prevent loss of sample whilst lyophilization put on the squares and fix with a rubber band. Close the centrifuge tube and put your samples in the -80°C freezer at least 2 hours.

Remove the caps from the tubes, place the tubes in the lyophilizer overnight.

Preparing for kit

1. Resuspend the dried protein in 25 µl loading buffer.
2. Take one 1 µl of the sample and add 19 µl loading buffer in a fresh reaction tube.
3. Dilute 1 milligram BSA in 2 ml.
4. Standard curve: Calculate the volume for the different BSA-concentrations (as shown on the table) in each well.
5. Protein of interest: Based on your expectations calculate the volume of the 1:20 dilution of your protein for the assay.
6. Use the kit as described in the instruction.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,5	0,5	0,5									
B	1	1	1									
C	2	2	2									
D	4	4	4									
E	5	5	5									
F	8	8	8									
G	10	10	10									
H	B	B	B									

Green: BSA µg, White: Blank (Mixture of reagent a and b from the kit), Red: proteins of interest + protein for comparison

SDS-PAGE & Western Blot

1. Perform SDS-Page as described in Protocol *SDS-PAGE*. Load different amounts of your resuspended protein and the protein with the known secretion rate.
2. Perform western blot.
3. Measure your signal via ImageJ and create a standard curve with your protein with the known secretion rate.
4. Calculate the amount of secreted protein (excel sheet).