Screening

Solutions & Materials

2x SDS loading buffer		
Ingredient	Volumes	Final concentration
1 M Tris pH 6.8	600 µl	60 mM
Glycerol	5 ml	50%
20% SDS	1 ml	2%
Bromphenolblue	small tip of spatula	-
1 M DTT	1 ml	100 mM
H_2O	2,4 ml	-
Final	10 ml	-

Other materials: acetone, 95% ethanol, Milli-Q water, rubber bands, transparent nylon thighs

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

Protein precipitation (desalting)

1. Take your samples out of the lyophilizer. Resuspend dried protein in 100 µl Milli-Q water or the compatible buffer for your analysis. Spin down for a few seconds to get the whole sample into the bottom of your tubes.
2. Transfer sample into a 2 ml reaction tube.
3. Add 6 times the amount of acetone to your sample (600 µl).
4. Incubate 20 minutes at -80°C.
5. Centrifuge samples at high speed (25.000 g) at 4°C for 30 minutes.
6. Remove the supernatant. Be careful, don't remove the sediment.
7. *Optional washing step*: Add 500 µl 95% Ethanol and centrifuge 10 minutes, 25.000 g at 4°C.
8. Place your samples with opened caps under the hood to dry the pellet.

Loading preparation

1. Resuspend protein pellets in 2x SDS loading buffer. (Volume depends on the desired concentration)
2. Denature the samples by incubating 1 min at 95°C.
3. Run SDS-PAGE (see Protocol *SDS-PAGE*)