Immunoblot

Preparing the cells for an immunoblot:

1. For analysis of intracellular expression

- centrifuge **1 ml cell culture** at 3000 g for 5 minutes
- remove supernantant
- resuspend the cell pellet in 0,2 ml of gel loading buffer **(lämmli 5x)**
- boil samples at **97°C for 5– 10 minutes**
- after cooling, store the pellet at –80°C

2. For analysis of secretory protein expression

- centrifuge **10 ml cell culture** at 3000 g for 10 minutes
- remove supernantant
- add 2 ml of **50 % TCA** to 8 ml of the supernantant
- leave on ice for 30 minutes
- centrifuge the samples at **15000g for 15 minutes**
- remove supernantant
- wash the pellet with 1 ml f **80% aceton**, centrifuge the samples at 15000g for 15 minutes and remove the supernant
- resuspend the cell pellet in 80 µl of gel loading buffer **(lämmli 5x)**
- boil samples at **97°C for 5– 10 minutes**
- after cooling, store the pellet at –80°C

Preparing Immunoblot:

- before loading the gel, boil the samples for 10 minutes
- load the gel with 5 µl ladder and 20 µl of the samples an run **(1– 1:30 hours; 600mV, 100W, 20mA)**

Preparing the westernblot:

- Transfer gel to a membrane and let them run **(1– 1:30 hours; 100V; 100W; 20mA)**

Preparing ponceau staining:

- wash membrane in water
- add ponceau solution to stain the proteins
- remove the ponceau staining and was the membrane with water
- wash the membrane a few times with TBS buffer (→ Basic Protocol: Buffer TBS)
- wash the membrane with milk **(add 2,5g milkpowder in 50ml TBS)** → until the membrane is not longer red

Antibody staining:

- put primary antibodys and milk on the membrane for **1 hour**
- wash the membrane three times for 10 minutes in TBS buffer (→ Basic Protocol: Buffer TBS)
- put secondary antibodys and milk on the membrane for **1 hour**
- wash the membrane three times for 10 minutes in TBS buffer