# Protein detection



## Supernatants

- 24 h after tranfection, cell medium was collected and placed in microcentrifuges.
- After centrifugation (1 min/max rpm) the medium was mixed with cooled methanol (1:4) and frozen over night at -80°C.
- After 24 h the mixture was centrifuged for an hour at 4°C (max rpm).
- Methanol was removed by pippeting and heating the samples at 50°C for 15 minutes.
- The samples were resuspended in 45 µl of PBS buffer. BCA test was performed in order to determine the concentration of proteins in the sample.

## Cell lysates

- After supernatant removal, the cells were first washed with 1 ml PBS buffer and resuspended in 100 µl of RIPA buffer with added protease inhibitors.
- The samples were left on 95°C for 15 minutes and then sonicated for two minutes (2s ON, 1S OFF, 35% amplitude) and incubated over night at -80 °C.
- After 24 h samples were resuspeded and spinned down before BCA test was performed.

## Protein concentration measurement- BCA test

BCA test was performed acording to instructions of The Thermo Scientific™ Pierce™ BCA Protein Assay. The test is based on reduction of $Cu^{2+}$ from the copper (II) sulfate to $Cu^{+}$ in a temperature dependent manner. The amount of reduced $Cu^{2+}$ is proportional to the amount of protein present in the solution. Next the two molecules of bicinchoninic acid chelate with each $Cu^{+}$ ion , forming a purple coloured complex that strongly absorbs light at 562 nm.

## Western blot

- Samples were loaded on 12 % acrylamide gel and ran at a constant voltage (200 V) for 45 minutes. The amount of loaded sample was determined with BCA test. Gels were then washed with distilled water.
- The proteins were transferred to a nitrocellulose membrane at a constant current (350 mA) for 1.5 h. Membranes were blocked for an hour (or overnight) by incubation in I-Block blocking solution (Thermo Fisher).
- Used proteins had AU1, HA, Myc or Flag tag at C- or N-terminal end of the protein. Membranes were incubated with corresponding primary antibodies (anti- AU1, HA, Myc or Flag tag), diluted (1:1000) in I-Block for 1.5 h at room temperature and 200 rpm. Membranes were washed in

wash buffer three times for 5 minutes. Membranes were then incubated with secondary antibodies conjugated with HRP, for 45 minutes at room temperature and 200 rpm. HRP activity was detected by addition of SuperSignal West Femto or Pico Substrate (Thermo Scientific). Images were captured with G:Box imaging system (Syngene).