

## Solutions to prepare

H <sub>2</sub> O for mass spectrometry (MS water)
200 mM Dithioerythritol
500 mM Iodoacetamide
Guanidine hydrochloride 6M, K <sub>2</sub> HPO <sub>4</sub> 50mM, pH 7,4 – 7,8
50 mM NH <sub>4</sub> HCO <sub>3</sub>
Bradford reagent (diluted 5X)
1 µg/µL Bovin gamma globuline (BgG)
Trypsin
0.5% formic acid in MS water

## Protein extraction :

1. If necessary, wash cells with 1 mL of PBS 1X
2. Centrifuge samples at 10,000 rpm for 10 minutes and discard the supernatant
3. Add 50 µL of guanidine hydrochloride to small pellets and 100 µL to larger pellets
4. Perform ultrasonication on the samples (pulse 1, amplitude 20%) for three cycles of 10 seconds each and place the samples on ice between each cycle
5. Centrifuge samples at 10,000 rpm for 10 minutes
6. Collect supernatants and store at -20°C

## Protein dosage:

In a 96 well plate:

1. Prepare the standard curve with different dilutions of the BgG in triplicates:

BgG	Water
10 µL (from 0.1 µg/µL)	15 µL
20 µL (from 0.1 µg/µL)	5 µL
10 µL (from 0.5 µg/µL)	15 µL
16 µL (from 0.5 µg/µL)	9 µL
10 µL (from 1 µg/µL)	15 µL

2. Dilute the samples if necessary
3. Add 2 µL of samples and 23 µL of water to each well, in triplicates
4. Add 225 µL of diluted Bradford reagent to each well
5. Measure the absorbance at 595 nm
6. Plot the OD values and the standard protein concentrations
7. Determine protein concentration of samples (multiply by the dilution factor) by using the standard curve

## Reduction:

1. Collect 50 µg of proteins and adjust the volume with guanidine hydrochloride to reach the volume of the least concentrated sample

2. Add 1  $\mu\text{L}$  of Dithioerythritol for 20  $\mu\text{L}$  of sample
3. Vortex briefly and incubate for 20 minutes at  $56^\circ\text{C}$  and 450 rpm

***Alkylation:***

1. Add 1  $\mu\text{L}$  of Iodoacetamide for 21  $\mu\text{L}$  of sample
2. Vortex briefly and incubate for 30 minutes at room temperature, in the dark

***Protein precipitation:***

1. Dilute samples 2X with MS water
2. Add 4 times the sample volume of cold acetone to each sample
3. Incubate for a minimum of 1 hour at  $-20^\circ\text{C}$  (overnight is recommended for better protein precipitation)
4. Centrifuge at 13,000 rpm for 20 minutes at  $4^\circ\text{C}$
5. Discard the supernatant and let the residual acetone evaporate for maximum 10 minutes.

***Trypsinolysis:***

1. Prepare a trypsin solution of  $0.05 \mu\text{g}/\mu\text{L}$  with the  $\text{NH}_4\text{HCO}_3$  buffer (50mM)
2. Add 20  $\mu\text{L}$  (1  $\mu\text{g}$ ) of trypsin to digest 50  $\mu\text{g}$  of proteins (avoid making bubbles)
3. Incubate overnight at  $37^\circ\text{C}$  at 450 rpm
4. Stop trypsinolysis with 5  $\mu\text{L}$  of a 0.5% formic acid solution
5. Keep the samples at  $-80^\circ\text{C}$  for long-term storage and  $-20^\circ\text{C}$  for short-term storage
6. Before injection, centrifuge the samples at 13,000 rpm for 20 minutes