Tryptic cleavage in the gel with different proteases

(Trypsin, Arg-C, Asp-N, Lys-C, Glu-C)

To wash

- Discard the liquid
- 10min 60µL 50% ACN / 100mM TEAB
- 10min 60µL ACN
- 10min 60µL 100mM TEAB
- 10min 60µL ACN
- 10min 60µL 100mM TEAB
- 10min 60µL ACN

The liquid is discarded after each step.

Reduction and alkylation

- 60µL 10mM DTT (in 100mM TEAB) → 57 °C, 30min
- 10min 60µL ACN
- 60µL 10 mM IAA (in 100mM TEAB) → 25 °C, 20min, dark
- 10min 60µL ACN
- 10min 60µL 100mM TEAB
- 10min 60µL ACN
- 10min 60µL ACN

The liquid is discarded after each step.

Cleavage

- Add 200ng of the appropriate enzyme in 40µl 25mM TEAB. Use different buffer for Arg-C: 100mM Tris-HCl pH 8, 10mM $CaCl_2$, 5mM DTT, 0,5mM EDTA
- Incubate on ice for 1h
- Incubate with Trypsin, Arg-C, Asp-N and Lys-C at 37°C, with Glu-C at room temperature (temperature should not exceed 25°C) over night
- Collect now liquid after each step
- Add 20µl 0,1% TFA
- 30min 30µl 0,1%TFA/ACN
- 20min 20µl ACN
- 20min 30µl 100mM TEAB for Samples digested with Arg-C 100mM Tris-HCl pH8
- 20min 20µl ACN
- 20min 20µl ACN: Put Samples in SpeedVac to dry.