

## Methanol / Chloroform Extraction for Proteins

### Guidelines for sample preparation

(How to protect your samples from contamination with keratin)

- Clean your bench
  - Try to avoid contact of samples and solutions with dust, skin or hair
  - Wear gloves at all times
  - All reagents should be prepared fresh
  - Use ultra-pure water for all solutions
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- Use **200 µl** of sample. If sample volume is bigger than 200 µl, split into multiple tubes. If sample is less than 200 µl bring the volume up to 200 µl with MilliQ-H<sub>2</sub>O.
  - Add **600 µl** methanol
  - Add **150 µl** chloroform
  - VORTEX
  - Add **450 µl** MilliQ-H<sub>2</sub>O
  - VORTEX
  - Centrifuge (max. speed, table top centrifuge) at room temp. for 1 min\*.
  - Pipette off upper aqueous phase without disrupting the precipitate at the interface.
  - Add **450 µl** methanol to the sample containing the organic phase (with precipitate)
  - VORTEX
  - Centrifuge at room temp. for 2 min.
  - Remove supernatant
  - go to "IN-SOLUTION DIGESTION" protocol

\* Centrifuge longer if still turbid