## **KESSLER LAB-PROTEOMICS PROTOCOLS**

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## **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
- 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>0.
- Add **600 µI** methanol
- Add **150 µl** chloroform
- VORTEX
- Add **450 µI** MilliQ-H<sub>2</sub>0
- 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 µI** 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