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-H₂0.
- Add **600 µI** methanol
- Add **150** µl chloroform
- VORTEX
- Add 450 μl MilliQ-H₂0
- 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