Recommended Protein Extraction Buffer

1. RIPA buffer (extract proteins for in-gel digestion)

   50 mM Tris-HCl (pH 7.4)
   150 mM NaCl
   1% NP-40
   0.25% Na-deoxycholate
   Protease Inhibitor Mix (e.g. 1 mM PMSF, 10 ng/mL leupeptin, 1 ng/mL aprotinin)
   SDS 0.1 - 2% (optional, e.g. 2% for membrane proteins)

2. RIPA buffer (extract proteins for in-solution digestion)

   50 mM Tris-HCl (pH 7.4)
   150 mM NaCl
   0.5% RapiGest SF (Waters)
   0.25% Na-deoxycholate
   Protease Inhibitor Mix (e.g. 1 mM PMSF, 10 ng/mL leupeptin, 1 ng/mL aprotinin)
   SDS 0.1 - 2% (not recommended, required further SCX-cleanup procedure)

3. HEPES buffer (extract proteins for dimethylation/iTRAQ/TMT labeling)

   20 mM **HEPES** (pH 7.4)
   150 mM NaCl
   1 mM EDTA
   0.5% SDS
   Protease Inhibitor Mix (e.g. 1 mM PMSF, 10 ng/mL leupeptin, 1 ng/mL aprotinin)

4. HEPES buffer (extract proteins for phosphoproteins, compatible with iTRAQ/TMT labeling)

   20 mM **HEPES** (pH 7.4)
   1 mM EDTA
   0.1% SDS
   1 mM Glycerolphosphate
   1 mM Na_3VO_4
   2.5 mM $Na_4P_2O_7$
   Protease Inhibitor Mix (e.g. 1 mM PMSF, 10 ng/mL leupeptin, 1 ng/mL aprotinin)

Suggested Protein Extraction Protocol

Before starting:

Protocol for protein extraction must be adjusted for the proteins of interest as well as the following assay to be run. The detergents, such as SDS, NP-40, Triton X-100, Tween 20 and CHAPS, are usually used to increase the yield of protein extraction. Unfortunately, high concentration of detergents would strongly reduce the efficiency of protease (trypsin) digestion and peptide identification by mass spectrometry. If the use of high concentration of detergent can't be avoided, a following clean-up procedure or alternative MS-compatible detergents (e.g. RapiGest SF, PPS Silent Surfactant, ProteaseMAX and Invitrosol) were strongly recommended for maximum efficiency of protein identification.

Protein extraction for cultured cells

- Using 100 µl of an appropriate lysis buffer per $1x10^6$ - $1x10^7$ cells.
- Prior to use, add protease inhibitors to the ice-cold cell lysis buffer

For monolayer cells:

1. Rinse the monolayer cells 3 times with PBS. After the final rinse, use trypsin or a cell scraper to collect the cell and transfer the cell suspension to a tube. Centrifuge cells at 1,500 rpm for 10 min at 4°C and aspirate as much supernatant as possible. Proceed to step 3.

For suspension cells:

2. Centrifuge suspension at 1,500 rpm for 10 min at 4°C and aspirate the supernatant. Resuspend the pellet in cold PBS, transfer to a tube and centrifuge at 1,500 rpm for 10 min at 4°C. Aspirate the supernatant. Repeat 2 more times. Proceed to the step 3.
3. Add ice-cold cell lysis buffer and resuspend the pellet. Incubate on ice for 10 min.
4. Vortex tubes briefly and proceed to sonication cycle: 10 sec ON/10 sec OFF on ice. Repeat 3 – 5 cycles.
5. Transfer the supernatant to a new tube and centrifuge samples at 14,000 rpm for 15 min at 4°C to remove any remaining insoluble material.
6. Take an aliquot for the quantification and the further analysis if needed. Store protein extracts at -80°C.