- **RIPA buffer**
  50 mM Tris-HCl (pH 7.5)
  150 mM NaCl
  1% Triton X-100
  0.5% sodium deoxycholate
  0.1% SDS
  5 mM EDTA

  Store at 4°C and just before using add:
  10 μg/ml aprotinin (from 10 mg/ml stock in water; stored at –20°C)
  10 μg/ml leupeptin (from 10 mg/ml stock in water; stored at –20°C)
  1 μg/ml pepstatin (from 1 mg/ml stock in MeOH; stored at –20°C)
  1 μg/ml microcystin-LR (from 1 mg/ml stock in EtOH; stored at –20°C)
  0.2 mM activated Na_3VO_4 (from 200 mM stock prepared as described below; stored at –20°C)
  1 mM PMSF (from 100 mM stock in isopropanol; stored at –20°C)

  - *RIPA is a good general-purpose lysis buffer for whole-cell extracts of nuclear and cytoplasmic proteins*
  - *Most proteins are partially denatured in RIPA and most protein-protein complexes are disrupted*

- **NP-40 buffer**
  50 mM Tris-HCl (pH 8.0)
  150 mM NaCl
  0.5% NP-40 substitute
  5 mM EDTA

  Store at 4°C and just before using add:
  10 μg/ml aprotinin (from 10 mg/ml stock in water; stored at –20°C)
  10 μg/ml leupeptin (from 10 mg/ml stock in water; stored at –20°C)
  1 μg/ml pepstatin (from 1 mg/ml stock in MeOH; stored at –20°C)
  1 μg/ml microcystin-LR (from 1 mg/ml stock in EtOH; stored at –20°C)
  0.2 mM activated Na_3VO_4 (from 200 mM stock prepared as described below; stored at –20°C)
  1 mM PMSF (from 100 mM stock in isopropanol; stored at –20°C)

  - *NP-40 buffer is a good option for cytoplasmic proteins and readily extractable nuclear proteins*
  - *NP-40 buffer can be used for immunoprecipitation and co-immunoprecipitation studies*

- **DSP crosslinking buffer**
  40 mM HEPES (pH 7.5)
  120 mM NaCl
  1% Triton X-100
  1 mM EDTA
  10 mM β-glycerophosphate
  50 mM NaF

  Store at 4°C and just before using add:
  10 μg/ml aprotinin (from 10 mg/ml stock in water; stored at –20°C)
  10 μg/ml leupeptin (from 10 mg/ml stock in water; stored at –20°C)
  1 μg/ml pepstatin (from 1 mg/ml stock in MeOH; stored at –20°C)
  1 μg/ml microcystin-LR (from 1 mg/ml stock in EtOH; stored at –20°C)
  0.2 mM activated Na_3VO_4 (from 200 mM stock prepared as described below; stored at –20°C)
  1 mM PMSF (from 100 mM stock in isopropanol; stored at –20°C)
  0.8 mg/ml DSP (from 80 mg/ml stock freshly prepared in DMSO)

- *DSP crosslinking buffer is a good option for coimmunoprecipitations with weakly interacting proteins*
- *DSP crosslinking buffer must be prepared at room temperature to keep the high concentrations of DSP in solution during the crosslinking*

- **200 mM activated Na_3VO_4**
  1. Dissolve 368 mg of sodium orthovanadate in 9 ml purified water in a 50-ml conical tube and mix by vortexing
  2. Adjust the pH to 10 using either 1 N NaOH or 1 N HCl, with stirring. The starting pH of the sodium orthovanadate may vary with lots of the chemical. At pH 10, solution will be yellow.
  3. Boil solution until it turns colorless (approximately 10 min). All of the crystals should dissolve.
  4. Cool to room temperature.
  5. Readjust the pH to 10 and repeat steps 3 and 4 until solution remains colorless and pH stabilizes at 10. Adjust the final volume to 10 ml with purified water.
  6. Store the activated sodium orthovanadate in 500 μl aliquots and freeze at -20 °C.