**ITC sample preparation guideline**

**All samples and buffers should be filtered by 0.2 µm filter and degassed**

**Buffers**

- Buffers for macromolecule (M) and ligand (L) must be identical to minimize ΔH from dilution.

- If both M and L are large, dialyze simultaneously into the same buffer using separate dialysis vessels.

- If L is too small to be dialyzed, use the “dialysate” to dissolve solid ligand.

- Reducing agents: DTT is not recommended; substitute with β-mercaptoethanol or TCEP (not compatible with phosphate buffer) if possible.

- To minimize artifactual heats, the buffer should have a low enthalpy of ionization (e.g. phosphate, citrate, acetate).

- If you are working with synthetic peptides or oligonucleotides, be sure they are desalted prior to suspension in ITC buffer. Residual chemicals from synthesis (e.g., TFA and salts) will cause a buffer mismatch and high heats of dilution.

- A difference in [DMSO] between M and L will yield large ΔH from dilution and obscure binding data. If you use DMSO to solubilize a ligand, you will need to add DMSO to the macromolecule solution to match the concentration of the ligand solution. Many proteins are stable in the short term in up to 2-5% DMSO.

- Add the DMSO to the protein solution immediately before running the ITC experiment

**SAMPLES**

- Sample volume (Auto-iTC200)
  
  - Sample cell (Macromolecule) → 430 µL per run;
  - Syringe (Ligand) → 150 µL per run

- Sample concentrations

  Typical starting concentrations are 30-50 µM for the sample cell. Generally, the syringe concentration is 10x higher than the concentration in the sample cell; 7x higher for very tight binding and 10-15x (or more) for very weak binding.