Sample Preparation. Proper sample preparation is essential for successful ITC testing. The guidelines below must be strictly followed to insure an accurate ITC measurement of stoichiometry (n), heat of binding (ΔH), and binding constant (K_B or dissociation constant $K_D = 1/K_B$).
Both binding components **must be soluble** in buffer and **they must be stable 2-3 days stored at 4°C**.

- *The macromolecule solution* (the sample to be placed in the reaction cell): Require at least **0.40 ml** for one titration. ***If the binding parameters (K_D and 'n') are unknown the volume of sample must be sufficient for 2-3 titrations.*** The minimum recommended concentration is 5 μM, for tight binding where K_D is between 1 μM and 1 nM, and there is a large heat change. For weaker interactions, the macromolecule concentration should be at least 5 times K_D. Higher concentrations may be needed if ΔH is low. The macromolecule solution **must be** completely dialyzed against desired buffer. ***Please refer to the experimental design software to calculate exact conditions for your system of study.***
- *The ligand solution* (the sample to be placed in the injection syringe): Require at least **0.14 ml** for one titration. The optimum ligand concentration depends on binding parameters *(K_D and 'n')*. ***If the binding parameters (K_D and 'n') are unknown the concentration and volume of ligand solution must be sufficient to be diluted for 2-3 titrations*** (~ 0.3-0.5 mL at ligand concentration which is 20 times the macromolecular concentration). The lowest recommended ligand concentration is 50 μM. The ligand must be prepared in exactly the same buffer as the macromolecule. If the ligand is another macromolecule it must be dialyzed in the same buffer.
- *DTT should be avoided* as a reducing agent and replaced by TCEP (< 2mM) or β-mercaptoethanol (<5 mM).
- *Glycerol and other additives* which add viscosity to solution should be kept to a minimum, recommended final concentration **no more than 10%** (v/v).
- *Organic additives, such as DMSO,* in the final ligand solution should be kept as low as possible, recommended final concentration 1 to 2 %, **no more than 5%** (v/v).
- *The macromolecule and the ligand solutions* **must have** the exact same buffer constituents at the same concentrations. Remember this includes **additives** that you may use to improve solubility of either the syringe components or the cell components such as organic cosolvents (i.e., DMSO), or detergents.
- *Check the pH* of each solution after preparation. The difference has to be no more than 0.05 pH.
- *Regardless of whether or not any particles are visible* in your solutions, all syringe and cell samples must be filtered with a 0.2 micron filter.
- *The concentrations of both solutions* should be accurately determined after final preparation. Accurate determination of binding parameters is only possible if concentrations of binding components are accurately known.
- *The **20 mL** of matched buffer* is required for ITC experiment.
- *Use of metal ions other than Na^+ or K^+*: please inform core personnel before use. Special cleaning protocol is required for certain metal ions.
- *Use of organic solvents other than DSMO or alcohol*: please ask core personnel to determine compatibility.