

INSTRUCTIONS FOR USING THE VP-ITC at Ship Street 8/1/06

Always clean the instrument after you used it. Microcal suggest using a 5-10% Contrad-70 solution (<http://www.deconlabs.com/cleaning/contrad70.htm>). We have used Contrad70 and Liquinox and both seem to work fine. Fisher carries both of them.

Cleaning the ITC:

1. Turn on the ITC power. Then open VP VIEWER on the computer.
2. Remove the cap from the sample cell, use the long tipped syringe to remove the dH₂O from the sample cell.
3. Insert chamber flushing needle into sample cell.
4. Connect chamber flushing needle to injection syringe and flask via tubing.
5. Press [open fill port] in the command screen.
6. VERY CAREFULLY place injection syringe tip into dH₂O. Turn on the vacuum and run ~50ml.
7. Run ~50ml 5-10% CONTRAD-70 through injection syringe and chamber.
8. Run ~100ml of dH₂O through the injection syringe and chamber.
9. Rinse the flea stir bars
10. CAREFULLY remove dH₂O and allow air to run through the system for 5 minutes, or until injection syringe is dry.
11. Drain sample cell of dH₂O
12. Rinse chamber with reaction buffer.

Sample prep:

1. Load samples into degasser, put smaller stir bar in injection solution and larger stir bar in sample cell solution.
2. Turn on stirring, place lid and turn on vacuum. Use the metal screw on top of the lid to regulate the pressure. Degas until no more bubbles are seen.

Loading ligand:

1. Attach loading syringe to injection syringe.
2. Transfer the ligand solution to a small glass 6x50 mm culture tube.
3. Place ligand tube in tube holder and place injection syringe in tube.
4. Load syringe pulling slowly.
5. Press [close fill port] and remove loading syringe tubing.
6. Press [purge and refill].

Loading reaction sample:

1. Slowly draw up reaction solution into the long tipped syringe, avoid bubbles.
2. Insert long tipped syringe into sample cell until you feel the bottom. Raise tip ~1mm from the bottom.
3. Load sample slowly into the chamber avoiding bubbles.
4. Quickly dispense short blasts of sample into chamber to dislodge bubbles.
5. Rest long tipped syringe on black edge of the sample cell and remove excess sample.

Running experiment:

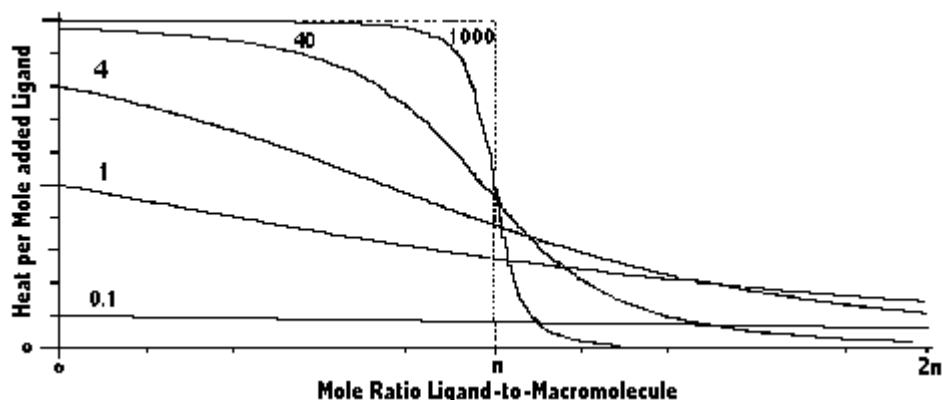
1. Remove injection syringe tip from ligand solution, and wipe gently with a kimwipe.
2. DO NOT BEND SYRINGE
3. CAREFULLY insert injection syringe into sample cell. Pop it into place.
4. Start the reaction by pressing [START]

End of experiment:

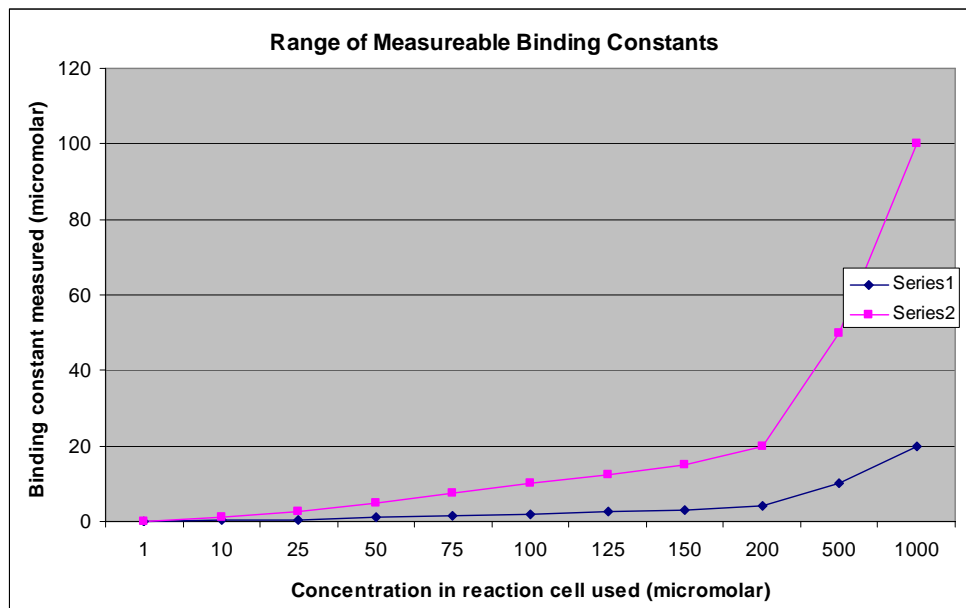
1. Remove injection syringe from reaction chamber. Recover reaction volume if desired.
2. Repeat cleaning steps #4 to #10.
3. Store the sample cell in dH₂O, replace cap.
4. Rinse all syringes in dH₂O.

Concentration Choosing Guide for VP-ITC

- a) The concentration in the injection syringe should be 10 times the concentration in the sample cell. This insures that for every molecule in the sample cell, two molecules are injected from the injection syringe.
- b) To choose a suitable concentration calculate the c value. This is the binding constant times the concentration that will be in the sample cell. To gain information from the ITC the c value should be between 10 and 100.



- c) In an exothermic reaction the above graph is vertically reversed. Bindings in the strong nanomolar range are difficult to measure, because of the small signal due to the low concentrations that have to be used.



Helpful Literature:

Pierce et al. *Isothermal Titration Calorimetry of Protein-Protein Interactions*. *Methods* 19, 213-221 (1999)

Perozzo et al. *Thermodynamics of Protein-Ligand Interactions: History, Presence, and Future Aspects*. *Journal of Receptors and Signal Transduction*. Vol. 24, Nos. 1 & 2, pp. 1-52, 2004