

# MicroCalorimeter ITC Directions

Turning the system ON:

1. Turn on computer and after Windows has loaded (password = carb), turn on the MicroCalorimeter VP-ITC unit.
2. Place the Injector Unit into the holder on the right side of the VT-IPC unit.
3. Rinse out cell: stored in water so remove the water with the glass syringe and long needle and then rinse with buffer (fill the syringe so that the meniscus is above 1.5 mL). Eject the sample slowly from 1.5 to 0.5 mL and then quickly to 0.0 mL to force air out of the unit.
4. Rinse out the reference cell: stored in water and then load buffer. (left hole in the sample area)
5. Load **sample** into the right hole using the syringe filled to 1.5 mLs (bottom of the meniscus).
6. Rinse out the Injector Unit: fill a vial with buffer and with Open Fill Port, pull buffer through with a syringe. Drain the buffer while in the Open Fill Port by removing the syringe.
7. Load **Ligand** into Injector Unit:
  - Hold eppendorf under the needle
  - Click Purge -> Refill: ligand should load into Fill Port
  - Click Open Fill Port: ligand flows back into eppendorf
  - While still Open Fill Port: use syringe to pull ligand into Port until the ligand is visible in the tubing connected to the syringe.
  - Click Close Fill Port: ligand loaded
8. Place Injector Unit into VP-ITC unit: stirrer goes into the sample hole.

Software:

1. Double click on <VPViewer> icon.
2. Cell 1 ITC
3. Thermostat Control
  - Make sure Jacket Temperature is the desired experimental temperature

4. Experimental Control

Total # of Injections	30	<u>Inject Parameters</u>	
Cell Temperature	25 deg C	5 µL	Volume
Reference Power	20 (µCal/sec)	10 sec	Duration
Initial Delay	60 s	240 sec	Spacing
Syringe Concentration	0.20 mM (ligand)	2	Filter Period
Cell Concentration	0.01 mM (sample)	Edit Mode: Apply to Rest	
Stir Speed	310		
Feedback Mode/Gain	High		
Datafile name	YOUR FILENAME		

5. ITC: Start Run

SPECIAL NOTE: ligand should be 10 – 20x the sample: Need ~ 500 µL to fill the syringe.  
RNA samples ~ 5 µM } Need 1.5 mL to fill the cell (at most 1.8 mL).  
Protein samples ~ 10 µM }

Data Analysis:

Double Click <Microcal Inc. ITC>

Read Data: C:\VpITC\Data

Window (on Menu Toolbar)

RawITC

Press Adjust Integrations to Integrate Individual: Necessary if baseline needs major correction

Click baseline, move peaks, return

Click Integrate

Only Integrates the region between the blue dashed vertical lines

Use >> to move to the next peak

Quit

Press Integrate All Peaks

Press Remove Bad Data

Model Fitting

One set of sites

If you modify fitting parameters, click Done to have the data shown in DeltaH window

Print DeltaH window

ITC (on Menu Toolbar)

Final Figure: shows the raw data with the fitted data (no parameters)