

## **Operating Instructions for the Leica Automatic Freeze-Substitution (AFS) Apparatus**

1. Plug in unit.
2. Set TF control to "O"
3. Raise glass plate and slide to open cover.
4. Insert LN<sub>2</sub> funnel with extension tube into central opening of substitution chamber and fill. Fill only until the top light on the LED is lit. (~40 liters).
5. Set the TF control to 50%.
6. Switch the instrument on.
7. Press <start>
8. Press <pause> and hold it depressed until three acoustic signals are heard AND the LEDs below <start> and <pause> are no longer lit.
9. Press number of the desired memory program or write a new program (see below).
10. Select proper specimen range tube according to program temperature.
  - a. Grey topped tube to -30°C
  - b. Black topped tube to -100°C
  - c. -140°C tube
11. Pre-cool reagents and load samples into aluminum containers.
12. Slide glass cover over samples and lower onto chamber.
13. Unscrew handle and retract to top position.
14. Press <start>
15. Change pre-cooled reagents and range tubes as necessary during run.
16. For Ultra-Violet polymerization:
  - a. Pre-cool appropriate sample holder and embedment media.
  - b. Transfer sample to holder and fill with fresh media
  - c. Seal sample container
  - d. Connect lamp to AFS and plug in
  - e. Exchange temperature range tube for red-topped temperature range tube
  - f. Slide glass cover over samples and push down onto temperature range tube.
  - g. Disconnect handle by rotating counter clockwise
  - h. Slide handled cover to back of unit
  - i. Plug n UV unit, place over chamber, and flip it's main switch to on
  - j. Select program on control unit and press start. The blue LED on the control unit should light up.

### **Programming the Memory**

1. Pressing <store> followed by one of the buttons 0 to 9 allows you to store up to 10 programs.
2. Press T1 button. While T1 light is lit select desired temperature by pressing + - button below temp display. Select time interval wanted for T1 by pressing + - button below time display. Repeat for T2 and T3.
3. Press S1 button. While S1 light is lit select the temperature increase with + - button below the temp display. If T1 < T2 and S1 must be positive. Repeat for S2.
4. Press <store> followed by the desired memory number. Press start.

*You must have completed a CMRF Project Information Sheet with the Business Office*