

Guideline (MANUAL) for operation of GC instruments in Abbott hall lab 338

IMPORTANT

- Replace N₂, H₂ or Air tank when the main pressure drops below 100 psi.
- Do NOT use matches or splints to light FID, use long-tip butane lighter in combination with ignitor when >150 °C, always verify there is a flame.
- The HYDROGEN, AIR and AUX GAS knobs should always be either full OFF (clockwise) or full ON (c-clockwise). Turn them gently! Do not force to open further!!!
- The column should NEVER be heated without a flow of carrier gas!!
- Always log yourself onto the log sheet.

Pressures & flow rates for HP5890 Series II with FID and capillary column^a

<i>N₂ (carrier gas)</i>	
<i>tank, 2nd stage</i>	80 psi
<i>inlet line</i>	60 psi
<i>column head</i>	~ 5 psi
<i>split vent</i>	30 mL/min
<i>purge vent (septum)</i>	3 mL/min
<i>column flow</i>	1 mL/min ^b

All the flow rates are preset, you need to verify them but DO NOT modify the settings unless instructed by your instructor. If you have any concerns contact Dr. Kubatova AH 301 7-0348

<i>FID gases</i>					
<i>Air</i>		<i>H₂</i>		<i>He</i>	
<i>tank, 2nd stage</i>	80 psi	<i>tank, 2nd stage</i>	40 psi		
<i>inlet line</i>	~ 40-50 psi	<i>inlet line</i>	~ 20-25 psi		
<i>FID flow</i>	400 mL/min ^d	<i>FID flow</i>	30 mL/min ^d	<i>aux. gas flow</i>	35 mL/min ^c

(a) This column head pressure is based on a 15 m x 0.25 mm ID capillary column.

(b) Control flow rate using column head-pressure valve.

(c) The AUX GAS control is an ON/OFF device combined with a needle valve for flow adjustment (center screw).

The needle valve is VERY delicate and should not be forced to either end of its travel.

(d) Control flow rate through column using inlet line-pressure valve.

Gas supplies:

Before starting make sure you have on all gas cylinders sufficient pressure. REPLACE N₂, H₂ OR AIR TANK WHEN THE MAIN PRESSURE DROPS BELOW 100 PSI.

Carrier gas: Nitrogen (carrier gas) ultra high purity.

Detector: Nitrogen, Hydrogen, Air (breathing quality)

Pressure regulators: the gas supplies are controlled with dual stage pressure regulators. The first stage shows the pressure in the gas tank (how much gas is still inside). Replace the gas tank when < 100 psi. The second stage shows pressure needed to supply gas to GC (note in this case enough for three GCs). Depending which GC will be operated, the on/off toggle valve has to be switched on.

CONDENSED MANUAL for the HP 5890 Series II GC's with FID

The following steps should be followed to start-up and shut-down each GC-FID system.

In case of maintenance, significant troubleshooting and repairs, consultation of the GC and integrator manufacturer operation and reference manuals may be needed.

A.	STARTUP OF INSTRUMENT.....	2
B.	OVERNIGHT SHUTDOWN OF INSTRUMENT	3
C.	LONG TERM SHUTDOWN OF INSTRUMENT.....	3
D.	OPERATION OF GC INSTRUMENT	3
D.1.	Sample injection into a split/splitless injection port.....	3
D.2.	Operating split/splitless injector port	4
D.3.	Temperature programming.....	5
D.4.	Stopwatch/Time	5
E.	OPERATION OF CLARITY LITE SOFTWARE.....	6
F.	OPERATION OF INTEGRATOR.....	7

A. STARTUP OF INSTRUMENT

1. Log any work, and pressure in gas tanks to the log sheets provided for each GC..
2. Turn ON the main valves for the N₂ (UHP grade), H₂, air, and N₂ cylinders. If the main pressure for any tank is less than 100 psi, it should be replaced.
3. OPEN the N₂, H₂, & air manifold valves (toggle on/off) to the GC (#1, 2, or 3) you will be using.
4. Note which injector/detector ports have the column installed (A-front or B-back) and *confirm the pressures and flow rates as shown in Table on 1st page*. Use the manual bubble meter to verify the flow rates (a stop watch on the instrument for determination is a convenient tool for the determination).

The flow rates are determined by connecting the flow meter to the outlets of detector (when detector is off and cold), split line, and septum purge. If the flow rates need to be reset, open completely the total flow control, set the column head pressure to achieve sufficient flow rate on the column; setup of the flow rate on the septum purge; and decrease the flow rate on split line by the total flow control.

5. Turn on the GC main power and set the heated zones to nominal initial temperatures (oven 35 °C, injector 250 °C, and detector 300 °C). Note this may differ depending on your actual work.
6. Configure the injector for split/splitless operation (see Section D.2 for programming) and ignite the FID using procedure described below:
 - a. Through the GC keypad, set DET A (or B) ON, set SIG1 to A (or B), and press SIG1 (twice). This displays the FID signal (in pA), *which should be less than 1 pA before ignition*.
 - b. Use a long-tip butane lighter to ignite flame. When the temperature of the detector reaches 150 °C, the detector may be ignited. To ignite the FID, turn the HYGROGEN fully ON, after 2-4 seconds proceed with AIR. Lower the butane flame to the collector opening, observe the FID signal to see response. Often pushing igniter button at the moment of igniting with butane lighter helps.
 - c. If the FID ignites, an audible pop may be heard. The signal will jump above 100 pA then quickly drop back to between **10 – 20 pA** and remain stable to within ± 0.1 pA (use a glossy

surface like an empty tube to verify there is a flame). Turn the AUX GAS fully ON, make sure that flame stays lit (based on the output signal and/or condensation on glossy surface).

7. Turn ON the computer and Clarity software.
8. Before running any samples, a blank run injection (1 μL solvent) using a gradient program from 50-300 $^{\circ}\text{C}$ should be performed. The chromatogram of this run should provide a clear baseline with increase at higher temperature (no peaks with exception of solvent). If this is not obtained the blank should be return or the personnel responsible contacted.
9. The system should be ready to operate at this point. The programming is operated from GC, the software is used only to record and save the chromatograms.

B. OVERNIGHT SHUTDOWN OF INSTRUMENT

The instrument can be left ON overnight. However, students **must inform** the instructor. To save the carrier gas the overall flow rate (split line) should be reduced to 5 ml/min. When reducing total flow, the pressure on the column should be checked in 20 min.

C. LONG TERM SHUTDOWN OF INSTRUMENT

1. Shut down GC in the following order:
 - a. Turn OFF the integrator.
 - b. For the FID, turn the AIR, HYDROGEN and AUX GAS fully OFF. The FID signal should drop to less than 1 pA.
 - c. Turn OFF the Injector and Detector heaters and set the oven temperature to ambient.
 - d. Wait for the entire system to reach room temperature, then, turn off the oven, and then turn off the GC main power.
2. CLOSE the He, H₂, & air toggle valves to each GC not used.
3. Turn OFF the main valves for the N₂ (UHP grade), H₂, and air cylinders if none of the GCs are used. If the main pressure for any tank is less than 100 psi, it should be replaced.

D. OPERATION OF GC INSTRUMENT

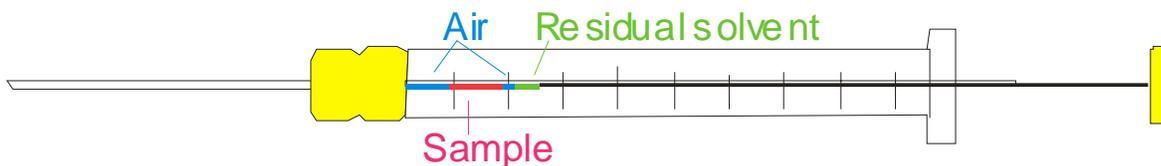
(Detailed instructions are available in the manufacturer manual)

D.1. Sample injection into a split/splitless injection port

The suitable volume of sample injected depends on the volume of the injector liner. The injector liner with i.d. 4 mm has a volume of $\sim 900 \mu\text{L}$. Do not forget that liquid expands when converted to the gas phase. The expansion depends on the pressure, temperature, density and MW of solvent used (defined by the ideal gas law). See equations in provided teaching materials, or you can use a vapor volume calculator provided by Agilent to determine the volumes.

Generally the 4 mm i.d. injector liner allows for injection of 1 μL for the majority of solvents. When making an injection, it is important to keep firm pressure on the syringe plunger or the injector pressure could cause it to blow off.

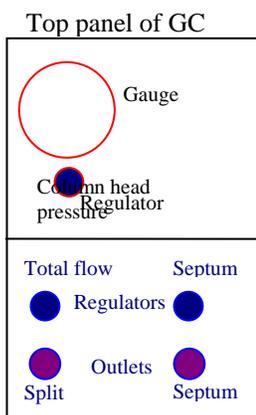
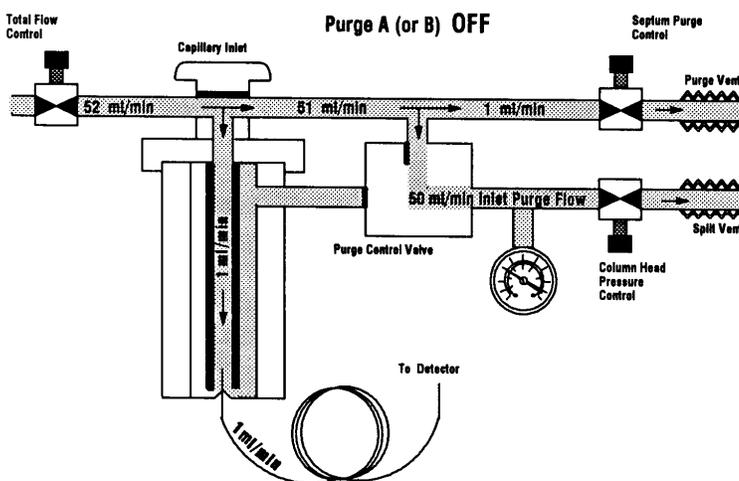
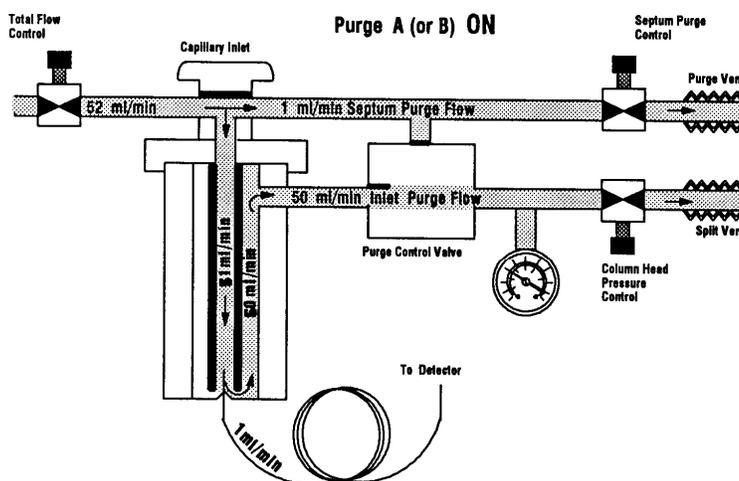
To ensure injection of the same volume, you need to preclean the syringe in the pure solvent, then draw 0.2 μL of air, followed by 1 μL of sample, and again air. This will result in a 1 μL band of the sample visible in the syringe.



The syringe is introduced into the injector, the piston pushed quickly, then the syringe is quickly withdrawn from the injector in one smooth upward motion. To start the program, push the start button on GC.

D.2. Operating split/splitless injector port

Always make sure you are operating the injector port in which column is installed (A–front or B–back). The diagrams below show the split and splitless flow diagrams. Three regulators on the top panel are setting the correct flow rates on the system. On the top panel, we can also observe gauge showing the head pressure on the column and two outlets one for split/splitless line and one for septum purge. Total flow control determines overall flow rate of Helium on the GC system (this include helium going through column, septum purge, split vent, and also makeup gas on detector).



Besides the setup of flow rates, purge control valve needs to be programmed. This valve controls whether the injection will be performed in split or splitless mode.

For split injection the valve has to be continuously set to “Purge ON” (See top figure for the gas line setup). Go to the electronic board on the front of the GC (left side).

Programming of split operation (turns purge valve A on)

PURGE/VALVE (A) (ON)

PURGE/VALVE (A) (TIME) (ON) (0) (ENTER)

PURGE/VALVE (A) (TIME) (OFF) (0) (ENTER)

For splitless injection the purge has to be off for the initial splitless time and than ON

PURGE/VALVE (A) (TIME) (OFF) (0) (ENTER)

PURGE/VALVE (A) (TIME) (ON) (1) (ENTER)

In this case the valve is off initially, and switched on at 1 min. **Always make sure the valve is off just before the injection.**

PURGE/VALVE (A) (OFF)

If you run more than one run, you can set purge time off just before the end of analysis.

D.3. Temperature programming

Single or multiple temperature ramp may be programmed; the highest rate is ca. **35°C/min**. If the instrument starts blinking red during the run, and the temperature gradient is higher, it is possible that the instrument cannot increase the temperature linearly.

ISOTHERMAL

OVEN TEMP (9) (5) (ENTER) Sets the oven temperature to 95 °C.

INIT TIME (1) (ENTER) Sets initial time to 1 min. This defines how long will be the analysis.

RATE (0) (ENTER) Eliminates all following temperature programs.

1 RAMP

OVEN TEMP (INIT VALUE) (3) (5) (ENTER) Sets initial temperature to 35 °C.

INIT TIME (1) (ENTER) Sets initial time to 1 min.

RATE (3) (5) (ENTER) Sets rate to 35 °C/min.

FINAL VALUE (9) (0) (ENTER) Sets final temperature to 90 °C.

FINAL TIME (5) (ENTER) Sets final time to 5 min.

MULTIRAMP

Input ramp 1 as shown above and then.

RATE (A) (5) (ENTER) Sets 2nd rate to 5 °C/min.

FINAL VALUE (2) (8) (0) (ENTER)

FINAL TIME (5) (ENTER) Sets 2nd final temperature and time to 280 °C for 5 min. Note the instrument should show you this is “A” final value and time. To program third ramp use program “B”.

D.4. Stopwatch/Time

Pushing button “Time” before run scrolls through a last run time, next run time, and stopwatch. During a run, it scrolls through the elapsed time, remaining time, and stopwatch.

You operate stopwatch by pushing “ENTER” and “CLEAR”.

E. OPERATION OF CLARITY LITE SOFTWARE

Acquisition

1. In the Windows Explorer create a directory to save your data this directory must be located in C:\ClarityLite\Data\The name of directory should employ the following format YYMMDD_II where II stands for your initials.
2. Open the Clarity Lite software located at the desktop
3. Click ok for “Administrator”
4. Go to “Analysis” menu and select “single analysis”
5. In the control window, label your sample: follow with the chronological order (e.g. “01hexane”, “02BTEX mix” etc.) and make sure your directly is correctly specified (use browse to find it)
6. Click on “ok,” the software should go to “waiting mode”
7. Wait until the GC is ready and inject your sample. Click on “Start” button on the GC. The software will start acquiring the data.
8. To view online the sample chromatogram, go to “Window”-> “Data acquisition”.
9. When the analysis is finished (the GC will automatically stop), in the software (Clarity Lite) go to “Analysis” menu -> “single analysis”and click on “Stop” button in the control window. The software will stop acquiring the data.
10. For a new sample injection, follow the steps 1-8.

Data Viewing/Processing

1. To view the acquired data go to “Window”-> “Chromatogram”.
2. To magnify the chromatogram, hold the left button of the mouse and drag across. To go back, double right click on the mouse.
3. If you have chromatograms in overlaid mode and you need to see a single chromatogram uncheck overlaid mode in file menu.
4. To specify integration parameters go to menu Chromatogram /Integration
5. To change the chromatogram display got to menu Display/Properties.
6. The integration data can be view upon selection the Result Table.
7. To copy the chromatogram to the MS Powerpoint, in the Chromatogram window go to “File” -> “Export” -> “Export as picture to clipboard”.
8. In the Powerpoint click “paste special” -> “enhanced metafile” -> select the object -> right click and select “ungroup”, repeat ungrouping and edit the chromatogram. Label the peaks and the chromatogram. At the end select all objects of the chromatogram and regroup them.
9. To print make sure the switch in right hand corner of the screen is on.

F. OPERATION OF INTEGRATOR

(Not currently in use, detailed instructions are available in manual)

Turn the integrator ON.

To check the major parameters press [LIST] [LIST] (twice)

Those parameters can be changed by selecting the [parameter] [value] [ENTER]

ZERO sets the baseline zero position

ATT2^ attenuation (-8 to 36). For the attenuation = 0 the plotter sensitivity is 1 mV full scale. Each step lower doubles the heights of peaks (e.g. lower number = bigger peaks).

CHT SP = chart speed in cm/min.

AR REJ = area reject, is a minimum value of the reported peak area.

THRSH = peak detection threshold, with range -6 to 28, defines minimum peak height that will be accepted.

PK WD peak width (default 0.04 min) if peaks are wider integrator will not recognize them.

Other parameters

Use [INTEG] and [number]

[INTEG][0] Set baseline now.

[INTEG][7] Turn off retention time labeling.

[INTEG][8] Turn on Start/stop marks (where integration stops starts).

[INTEG][9] Turn off integration.

Moving paper

[CTRL] [L] Advance paper to next top of form.

[CTRL] [A] Advance paper 1/8 of a line.

[ENTER] Advance paper one line.

[SHIFT]+[ENTER] Advances paper continuously.