

Manual Midas Autosampler.

- MAKE SURE NEVER TO:**
1. inject when the injector is dry.
 2. inject solutions containing particles.
 3. try to inject with an improper needle height adjustment. The needle will BREAK.

Ad.1.: Wash the injector with a suitable solvent before you start.

Ad.2.: Centrifuge all samples (and filtrate when necessary). Make sure there is nothing precipitating in your samples, especially when you cool them.

Ad.3.: Adjust the needle height so that it is correct for the vials you use. Have an experienced user help you if you do not know how to do this.

- Do make sure:**
1. You have enough eluent.
 2. The waste container where the eluent flows into is empty enough.
 3. There is enough wash solvent (see below).
 4. The needle height is set correct.

Always: wash the injector with a suitable solvent when you are about to stop. Finally wash the autosampler with 30% MeOH / 70% H₂O before switching it off (this will prevent microbial growth).

General info.

Most settings mentioned below should **not** be changed by the users. Since all settings are available to everybody there is no way to prevent faulty changes. Therefore: be sure what you are doing! The only settings that should be changed by the users are marked in red in the 'Programming Chart' (see last page).

The injector system works the same way as the handinjectors. Normally a 50 uL loop is installed. All volumes mentioned below apply only when this loop is used. With the 50 uL loop you can inject (= the amount going to the column) 1 - 50 uL sample.

The volume between the tip of the needle and the loop is approximately 25 uL (much more than for the handinjectors) which can be filled with wash solvent or reagent C (= the solvent in bottle C). The flush volume is normally set at 30 uL.

There are three types of injections possible:

- 1: full injection = 50 uL injected, the loop is overfilled 3 times ==> this uses 150 uL sample.
- 2: partial injection = 2 - 25 uL injected. The remaining loop volume (48 - 25 uL) is filled with eluent.
- 3: pick-up injection = 1 - 2 uL injected. The remaining loop volume (49 - 48 uL) is filled with reagent C, see below under point 6.

There are 3 types of vials available: 300 uL round bottom vials (for small amount or anaerobic injections, to be used with thin support sleeves), 500 uL eppendorf vials (for standard injections, to be used with normal support sleeves), and 1.5 mL vials (to be used when you want to mix reagent to the sample = for incubations).

	Vial		
	300 uL round bottom	500 uL eppendorf	1500 uL vial
approximate needle height	low	high	middle
flush volume uL	30	30	30
volume left in vial uL	15	75	200
uL required for 10 uL partial injection	55	115	240
uL required for 50 uL full injection	195	255	380

These are minimal volumes which apply only with the correct needle height!! Do use more sample when you have it.

Using the autosampler.

The enter knob [E] is used for insertion of values.

The [Escape] knob is used to go up one level.

1. Fill the wash bottle with a suitable solvent. Usually you will use the same solvent where you start your gradient with. Otherwise, use 5% MeOH + 95 % H₂O. **Degas the wash solvent** by sonification or flushing with argon.
2. Press '**WASH**'. This will wash the syringe, the tubing and the needle. Observe the syringe and check for airbubbles. When you see any, repeat the wash step. The volumes injected may not be accurate when airbubbles are left in the syringe.
3. **Connect the fluid lines:** a tube between the manual injector of the HPLC and the autosampler inlet (see figure inside the autosampler), the column to the autosampler outlet and the end of the column to the detector.
4. **Connect the (electrical) pulse wire** to the wire which is already in the detector.
5. Clean the manual injector and **switch the manual injector into the load position**.
6. Press 'Methods'. There are 8 methods available. Choose a number.

Proceed the autosampler programming as shown in the examples (do not start yet) up to the line 'Running the series'. Take the following into account:

- * Always use a flush volume of 30 uL.
- * When you want to add some reagent (e.g. 50 uL H₂O₂) to start a reaction then use the 'MIX' option: Select: 'Add' '50' uL from 'Reagent A' to 'sample'.
The minimal reaction time will be '1'1". Extra mixing is not necessary when you use the 1.5 mL bottles.
- * The '**Analysis time**' should be **1 min. longer then the run time** inserted in the HPLC program. This is because the chromatogram is calculated first before the system is ready for the next injection.
- * When you use the 'uL pickup' injection method the remaining loop volume (ca 48 uL) will be filled with reagent C (this is an extra 20 mL bottle which should be put into the tray). Fill bottle C with your starting eluent and place it into the tray.

7. Follow the HPLC manual to set up the system (like normal) up to the line:

Stop monitoring the baseline by **clicking the red injector button**.

When somebody has been using the system overnight with the autosampler, the detector is probably still switched on but the lamp is switched off. When you perform 'Setup' as described in the HPLC manual using method 'Meet', the lamp should lit after ca 30 sec.

The HPLC should be running at your starting flow + eluent composition before you proceed!

The text given below replaces the text in the HPLC manual under the heading '6. Injecting' when you use the autosampler.

8. In the 'Run Samples' screen of the HPLC program:

Select the 'Samples' tab. Click the left mouse button on the '**Method Set**' field and **select the method that has been set up for you** by an experienced user. Insert a sample name and run time. Check whether all settings are OK (remember that the RUN TIME in the HPLC program should be 1 min. shorter then in the autosampler, see above). **Copy the first line by clicking on the second line in the 'Sample Loading Table' for as many times as you have samples + 1 more. Change the 'Sample names'**.

The last run should be used to clean the column + the pump, decrease the flow and to switch off the lamp of the detector. **In the last line, select a suitable cleaning method (usually named STOP) in the 'Method Set' field. Change its function in the 'Function' field from 'Inject Samples' to 'Condition Column'.**

Select all runs by clicking and dragging the mouse over their numbers.

Click the Green Injector button.

Give your sample set a name, and select 'run selected' in the last window. The status will switch to 'injector wait' after a few seconds.

9. Place your samples in the tray of the autosampler. Press the 'Start/Stop' button, insert the start at series (usually #1) and stop after series number (usually #1). Press the 'START' soft button.

Off it goes. The status on the computer will switch to 'running'.