

Instructions for operating Cyttek automated microplate sampler (AMS)

(Generally any type of 96 well plate is ok, except for V bottom plates. The V bottom plates require an optional inverted mixing paddle, with its narrow end at the bottom. The AMS has been tested most extensively with Falcon, Nunc, and Corning round bottom polystyrene plates.)

These instructions for AMS operation should be followed closely – while the AMS unit and its AppleScript software are reasonably stable, they have a tendency to lock up when not operated correctly. When in doubt, turn off and on the AMS box, close the AMS program, and start over.

First...

- Turn on the cytometer as usual – Cyttek 20 I tank system first (blue box under the cytometer), then the cytometer itself, then the computer.
- U-bottom 96 well plates are best for microplate labeling and AMS acquisition. V-well plates will interfere with the blades of the mixer, and flat-bottom plates will not allow the cells to pellet. Labeling and analysis volumes should be 150 ul – plates can be centrifuged using plate holders at 400 g for 5 minutes to pellet cells between labeling and washings.
- You now need to do three things: (1) set up the AMS box, (2) set up the AMS program, and (3) attach the AMS robotic platform. These three things do not have to be done in any particular order – however, they must all be completed before a run can be carried out.

(1) Setting up the AMS box.

- Turn on the AMS box to the right of the cytometer. After an internal systems check, the green “Ready” light should come on, and **Change AMS Settings?** will appear on the AMS box screen.

The **Change AMS Settings?** menu lets you change the AMS operating characteristics, like the sample acquisition delay, boost time, mix time, etc. Most of this is already set up and should not be changed. However, you can indicate whether plates should be run in horizontal rows or vertical columns from this menu also, so you will need to enter this menu to change this. The row/column choice is unfortunately at the end of this menu, so you must navigate the other menu choices without changing anything to reach it.

When the screen queries you for a change, press **Yes** on the keypad to keep the old setting. If you want to change setting, press the **No** button, then enter the new setting, then press **Yes** to confirm.

- **Change AMS Settings? Press Yes**
- **Enter Mixing Time Press Yes**
- **Enter Boost Time Press Yes**
- **Enter Backflush Time Press Yes**
- **Enter Wash Time Press Yes**
- **Run by Columns?**

If you want to run by columns (vertically, as in A1, B1, C1, etc.), press **Yes**. If you want to run by rows (horizontally, as in A2, A2, A3, etc.), press **No**.

- **Cooler installed? Press No**

This selection accommodates a plate temperature control device we don't have, so press **No**.

- **Setup Mode? Press No**

This mode will cause the instrument to start sampling without connection to the AMS software; it is intended to let you set up your CellQuest template and settings from control samples in the microplate. We have found that it is easier to set up your control samples in normal FACS tubes and to the instrument setup prior to attaching the AMS for plate analysis. So, we recommend you press **No** here.

• **Another Block?**

The AMS box is now querying for the blocks of wells you want analyzed. You can specify up to 20 blocks of wells, which can be anywhere on the plate, in any order. The AMS screen will probably display **A1** as the initial well in your first block – if you want to start with this well, press **Yes**. If you want to change it, press **No**, then enter the correct well on the alphanumeric keypad. The first keypunch will be the well letter, and the second (and third for 10-12) will be the number. Once you enter the well designation, press **Yes**.

The AMS screen will then ask you for the ending well in the first block (the default value will probably be H12). Again, press **Yes** to select the default value, or **No** to change it, enter the correct value, then press **Yes**.

If you make any mistakes, the arrow keys on the keypad may allow you to back up a step. When in doubt, feel free to turn off and on the AMS box and start over.

• **Another Block?**

The AMS screen will then query you if you want any additional blocks (you can enter up to 20). Enter your additional blocks as indicated above, then press **No** at the next **Another Block?** message when you are done.

Some hints on setting up Blocks. Remember that you are already set up to run in either vertical columns or horizontal rows, and that the instrument runs in geometric blocks of wells, not sequentially. For example, if the AMS is set up to run in horizontal rows and you tell it to run wells A1 through B6, it will **not** run wells A1 through A12, then B1 through B6 – it will run A1 through A6, then B1 through B6. For partial plate analysis, you may need multiple blocks to run all the wells you want.

Once you press **No** after your final block selection, the following screen will appear...

Start AMS program

Press Go here,

Then Go on Mac

Load the Plate into the AMS. Be sure A1 is to the right rear of the table.

The AMS box is ready to run! **DO NOT PRESS GO** until the AMS software, AMS robotic platform and the cytometer are ready to run. The AMS box will hold at this point indefinitely until you are ready to run.

(2) Setting up the cytometer and AMS software.

The AMS program is loaded on the cytometer computer and can be started by clicking on the AMS alias icon located on the Desktop. The AMS software works by operating CellQuest using a Template and Instrument settings you have previously set up, and an Excel spreadsheet containing filenames for each sample, also set up prior to the run.

Setting up CellQuest. Open up CellQuest, and build your Template as usual (or call up an old one). The number of cells collected in each file will be taken from the Template, so make sure you have indicated this correctly (including the stopping gate). We also strongly recommend you set a time limit on your acquisition; you can do this in the Acquisition menu in CellQuest by selecting the “Number of events and time” option, and selecting a stop time, say around 2 minutes; this will prevent your well from going dry if your sample runs out prior to reaching the selected cell number. The AMS won't

automatically stop if a well runs dry. With a 6PSI run pressure you will aspirate approx. 1ul/sec of sample from your well. Therefore you should not let acquisition continue longer than 150 seconds for a well which has 250ul of starting volume. Also, enter the necessary Instrument Settings, or call them up from a settings file or from a previous sample file. It is at this point that you should run your control samples and confirm that your settings are correct; again, we recommend you do this in normal FACS tubes prior to attaching the AMS robotic platform to the instrument. When everything is ready, make sure your Template is saved, and that the desired Instrument Settings are currently loaded on the cytometer. Then close CellQuest (the AMS program will reopen it automatically once it is run).

Setting up Excel. Open MicroSoft Excel, and either open a previously prepared spreadsheet, or build a new one (A template is available on the website or in the AMS Folder on the desktop of the instrument). The AMS program will access this spreadsheet and enter the cell contents under the "Sample ID" field of each CellQuest data file. The Excel cells A1 through H12 correspond to the analyzed microtiter plate, so enter your chosen sample info into these cells. You can later specify that this Excel cell info also be incorporated into the filename as well as the Sample ID. If you just want to use the well designations (A1 through H12) as the Sample ID and/or filename one, a default spreadsheet is available in the AMS folder for this purpose (use the Excel 2001 version). The sheet number in the Excel workbook corresponds to the plate number you are running. Make sure if you are running a multiple plate assay there is a sheet in your workbook for every plate you plan on running. If you don't want to use any Excel information in either the filename or Sample ID field of your CellQuest files, you can use a blank spreadsheet; however, you absolutely need some sort of spreadsheet saved for the program to operate properly. When you are done, close Excel.

Setting up the AMS software. To operate the cytometer and save your files, the AMS software needs to know where Excel and CellQuest are, where your CellQuest Template is, and where you want your files saved. It also needs to know the communications mode to the AMS box, and how you want your files named. All of this information must be provided prior to the run. You must have a special Fileguard Log In Name in order to be able to access the software (See Mike or Joanne to obtain one).

Double-click on the AMS alias icon. The AMS menu will appear and will have four sections. The first part of the menu deals with **CellQuest...**

- **Location of CellQuest program.** This should already be entered; if it is not, click on the navigator and navigate to the CellQuest program (it should be in the BD Applications folder). Click **Choose** to set it.
- **Location of CellQuest Template.** This is the location of your particular Template. It has been found that storage of your Cellquest Template (or a copy of) in the AMS software folder provides better software stability. Navigate to it and click **Choose**.
- **Location of File Storage.** This is where your files should be saved. Navigate to your data folder and click **Choose**. You can create a new folder from here if desired.

The second part of the menu deals with **Excel...**

- **Location of Excel.** This should also be set up already. If it is not, navigate to it (in the Microsoft Office 2001 folder) and click **Choose**.
- **Location of Excel spreadsheet.** Navigate to it and click **Choose**. (There is a Template available for your use in the AMS folder on the desktop. You must use "save as" to save your information so as not to overwrite the template.)

The third part of the menu deals with the **computer connection to the AMS box...**

- Make certain that the **Belkin serial port adaptor** selection is chosen. The other available choices (modem port, Belkin printer adaptor) will not work. If the serial port choice is not available on the menu, make sure the Belkin adaptor (the blue/white plastic adaptor

sitting next to the AMS) is connected to both computer (via a USB cable) and the AMS box (via a serial cable), and that the red light on the adaptor is on. If the adaptor is connected and you still don't see it as a choice on the AMS menu, try unplugging the USB cable and replugging it in – the red light and a green chaser light will flash on the adaptor. Close the AMS program and reopen it. If you still don't see the Belkin adaptor choice, make sure the unit is correctly connected, shut down the computer, cytometer and AMS box and start up everything from scratch. If this still doesn't work, get Bill!

The fourth part of the menu deals with **designating the file naming convention**. On the left side of the menu you have several choices for filename and Sample ID designation. In all cases, the Excel spreadsheet information will be saved to the Sample ID field in your CellQuest data files; however, you can also have this information incorporated into the filename, as indicated below...

- **Well ID+Sample Info.** This choice will cause your filenames consist of the Well ID (A01 through H12), plus the Sample Info (whatever was entered into your spreadsheet), plus the plate number (usually 01), then the file suffix. So, if your Excel spreadsheet uses the word "control" for the first well, your first filename will look like...

A01control01.001

...where **A01** is the Well ID, **control** is the Excel spreadsheet Sample Info, **01** is the plate number and **.001** is the suffix.

- **Well ID.** This choice will only use the Well ID (A01 through H12) as your filename, plus the plate number, plus the suffix. So for the file above, the filename would be

A0101.001

...where **A01** is the Well ID, nothing is coming from the Excel spreadsheet, **01** is the plate number and **.001** is the suffix. In this mode, you can use a blank Excel spreadsheet, since no information is coming from it; however, you **MUST** have a spreadsheet designated, even if it is blank.

- **Sample Info.** This choice will only use the Sample Info coming from the Excel spreadsheet as your filename, plus the plate number, plus the suffix. So for the file above, the filename would be

control01.001

...where **control** is the Sample Info from the spreadsheet, **01** is the plate number and **.001** is the suffix.

- **Custom.** This choice allows you to designate a custom filename in the provided space that is independent of the Well ID or Sample Info. So, if "Bill1" were designated as the custom filename, the above filename would be...

Bill101.001

...where **Bill1** is the custom filename, **01** is the plate number and **.001** is the suffix. Since nothing is coming from the Excel spreadsheet in this mode, you can use a blank Excel spreadsheet as for the Well ID mode; however, you again **MUST** have a spreadsheet, even if it is blank.

- Also in the menu area are the **Plate ID** (which usually remains at 1 or 01) and the **Suffix** (illustrated as 001 above). You can use the typical applied sequential number sequence for the **Suffix** (001 through 096, as above) or the Well ID (A01 through H12).

Hints for file naming. Try not to create situations where duplicate file names occur! This is particularly easy in the Sample Info or Custom modes, since the Well ID (which is always different between samples) is not incorporated into the filenames. If a duplicate

filename situation does occur, the program will respond by inserting a letter (V through Z) at the end of the suffix. So, for example, the file after Bill101.001 would be Bill101.001V. However, this software feature is not stable, and you only have five duplicates this way (up to Z) – after this, the program will crash.

Finally...

- **Simulation.** Make sure this box is **unchecked**
- **Absolute Counts Module Present.** Make sure this box is also **unchecked**

The cytometer and AMS software are now ready to run.

(3) Setting up the AMS robotic platform.

The AMS robotic platform is located under the cytometer SIP tube. When the AMS unit is turned on, the platform will do a stepper motor test after the **Change AMS Setup?** menu is navigated through, or bypassed. When you are ready to run, hook the platform up to the cytometer.

- Slide the metal/black plastic sample holder on the cytometer to one side, and unscrew the black plastic conical collar holding the SIP tube on. Remove the collar and the SIP tube – this tube is in fact an outer jacket normally used for SIP tube washing and droplet containment. The inner tube should still be attached to the cytometer. Be very careful not to damage this thin metal tube. Put the outer SIP tube (and the BAL seal, if it is loose) somewhere safe!
- Attach the silicone tubing from the AMS platform to the exposed inner SIP tube on the cytometer – the silicone adaptor should slide easily over the SIP tube. Do this quickly, since the SIP inner tube will be dripping sheath buffer.
- Remove the threaded black plastic tube holder from the metal arm of the cytometer, and return the metal arm to its position under the SIP tube. The arm **MUST** be in this position for the instrument to operate correctly. The metal arm should not interfere with the AMS tubing.
- Insert your 96-well plate onto the robotic platform. Try not to disturb the position of the platform when doing this, since it has already undergone a position check during the AMS box setup. If you disturb the platform too much, the probe will not be centered on the wells and the AMS box will give an error message; turn off and on the box and set it up again if this happens.

Once you have set up the AMS box, cytometer, AMS software and robotic platform, you are ready to analyze.

- Go to the AMS box, where the following message should be on the screen.

Start AMS program

Press Go here,

Then Go on Mac

- Press **Go** on the AMS box. This should be done **BEFORE** pressing **Go** on the AMS software program.
- The AMS box screen should display...

Waiting for Mac

- Go to the AMS program on the Mac and press **Go**.
- The AMS should start analyzing immediately. The robotic arm will move the plate to the designated well; the sample probe will drop, seal the well, apply pressure and move the sample to the instrument. On the computer screen, you will see the AMS software open CellQuest, open the Excel spreadsheet, then move the CellQuest Template to the front of the Desktop. Once sample acquisition begins. Due to the length of the sample tubing, there will be a small delay in acquisition; this is reduced with an initial sample boost, which you will hear at the beginning of each sample

acquisition. The AMS box will place the cytometer in “Run” during each sample acquisition, and in “Setup” between samples.

- Once the sample begins to acquire, *watch the acquisition rate*. If it is too high, turn down the black knob in the front of the instrument slightly to reduce the sample pressure; conversely, turn it up slightly if the rate is too slow or acquisition is too delayed. The AMS box has its own air pump and bypasses the cytometer’s normal air pump, providing air pressure for the cytometer. This pressure (indicated on the gauge to the right of the black knob) should be no less than 5 psi and no higher than 7.5 psi. If the pressure is too high, the instrument may clog easily; less than 5 psi, and the sample won’t inject.

Software conflicts. If there are any problems with your AMS setup, the software will halt and an error message will appear. Follow the instruction and press **Go** on the software (not the AMS box) again. Once **Go** is pressed on the AMS box, the unit will wait about three minutes for a signal from the Mac, then time out. If this happens, repeat your AMS box setup when the **Another Plate?** message appears.

Instrument clogs. If the instrument attempts to acquire a well for more than 20 seconds with no events visible on CellQuest, the tubing or the sample probe are probably clogged. Press the **Done** button on the AMS box keypad, then press “**Pause**” and “**Save**” in the Cellquest acquisition window. The probe will raise and the robotic platform will move the probe over the wash well and the message “Backflush probe, Next Well=1, Redo well=2, Abort Run=3” appears. You should observe a drop every 6 seconds coming out of the tip at this point. If the rate is lower, there is a clog. Simply disconnect the silicone tubing (sample line) from the SIP tube on the cytometer. With the sample line disconnected from the cytometer, observe the drop formation rate at the tip of the cytometer’s uptake tube. If it is longer than a drop every 6 seconds, the sheath pressure is low. Check the sheath filter for air, and the cap of the sheath tank. If there is a drop every 6 seconds from the cytometer, then use the attached green syringe (no bevel on the needle) filled with PBS to clear the AMS sample line, then reconnect the line. Press the **1** on the keypad to continue to the next well, press **2** to redo the well, or **3** to abort the run. Pressing **2** will result in two data files being saved for the well. The second acquisition will have a “V” appended to the filename to distinguish it from the first data file. If clogs occur often, reduce the run pressure. The normal run pressure is 6 PSI, but it can be adjusted up to 7 PSI or down to 5.5 PSI. Remember, samples are being injected against sheath at 4.5 PSI, so do not adjust the sample pressure below 4.5 PSI, or no sample will be injected.

Stopping acquisition. If for any reason you want to stop acquisition prior to the completion of your plate, press **Abort** on the AMS software menu; this will stop data collection. On the AMS box, press **Done** to pause acquisition, then **Done** a second time to end the run; the **Another Plate?** message will appear in the AMS box. You can also simply turn the AMS box off and on again – the probe will by default lift off the well it is currently sampling.

Important! Although the AMS is fairly automated, it is not a completely “walkaway” system – keep an eye on it to watch for clogs, etc., and until you are completely comfortable with the block designation, filenaming etc. (to make sure that the wells you are analyzing truly correspond to what you think they are).

- When the plate is finished, the robotic platform will “park” the probe under the wash well, and the AMS will box will display the **Another Plate?** message. The AMS Software will similarly reset. If you wish to analyze another plate, press Yes, set up the block structure again like you did above, and make any changes to the AMS software press.

Then press **Go** on the AMS box, **Go** on the AMS software, and begin acquiring your next plate.

Shutting down the AMS.

- When you have finished all your plates, press **No** at the **Another Plate?** message. The AMS box will then display the **Save AMS Settings?** Message. If you choose **Yes**, all the settings you selected in the **Change AMS Settings?** menu (like row/column sampling) will be saved for next time. If you don't want to do this, press **No**.
- After saving (or not), the AMS box will guide you through the shutdown procedure for the AMS unit. The following message will be displayed...

For Shutdown

Vent Cytometer Sheath Tank

Then Press Done

- Since we have the 20 l tank system, the black pressure switch is inactive – loosen the sheath tank cap to depressurize the instrument. Then press **Done** for the next message...

Disconnect Sample Line From Cytometer

Then Press Done

- Just pull the AMS sample line off the end of the cytometer's sample uptake tube, and put it in the test tube on the AMS cover. The instrument will drain sheath buffer from the silicone tubing, and the next message will appear.

Go to Standby

Repressurize Sheath Tank

Then Press Done

- Put the cytometer in Standby and tighten the sheath tank cap. Replace the outer SIP tube correctly – make sure the SIP tube is inserted through the small O-ring on the inside of the black conical collar, and that the BAL seal is mounted spring side up. The reassembled SIP tube should reach to the bottom of a regular FACS tube. Then press **Done**. The next message is

Fill Wells With 5% Bleach, then DI water, Then Press Done

- Fill 4 wells of a column with 5% Bleach, and the remaining 4 wells in the Column with DI water, then press done. The probe then advances to the first specified well, and the message "**Well#A01**" appears, with the message "**Cleaning Press Done to Pause**". If Done is pressed during the cleaning cycle the probe goes to the wash port, and the message, "**Backflush Probe, Next Well=1, Redo well=2, Abort Run=3**" appears. Press 1 continues the cleaning cycle, pressing 2 reprocesses the well just used, and pressing 3 terminates the cleaning cycle.

To Restart Press Go

Or Turn Off the AMS

- You can now turn off the AMS, or press **Go** to restart. Once the AMS is off, clean and shut down the cytometer in the normal fashion.

These instructions were prepared by the Telford lab for the NCI ETIB Flow Cytometry Core Laboratory and its friends. 1-19-03 and modified (with permission) by J. Lannigan for use by UVa's Flow Cytometry Core customers.

