

# CHROMATOGRAPHY

# Integrating the CETAC MVX-7100 Autosampler with the NGC Chromatography System

Quick Guide

#### Introduction

This quick guide is designed to help with the execution of multiple automated small-scale injections using the NGC Chromatography System along with the Teledyne CETAC Technologies MVX-7100 Autosampler.

# **CETAC MVX-7100 Autosampler**

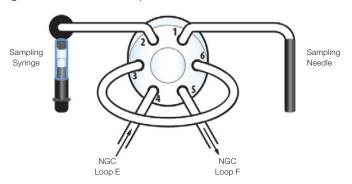
- Always store the instrument fluidics in 20% ethanol or another bacteriostatic solution
- Perform the following instructions in order and do not rapidly click through the software, otherwise errors will occur. If at any time you receive an error message or the software becomes unresponsive, close down the software and restart
- For further information or additional details about the CETAC MVX-7100 Workstation, contact Teledyne CETAC at 1-402-733-2829 or cetacservice@teledyne.com

# **Getting Started — Instrument Setup**

- 1. Ensure all cables required for the computer, NGC System, and power are appropriately connected.
- 2. Turn on the CETAC MVX-7100. There are three power switches, one on the back of each individual module.
- 3. Place the Rinse Station Pump Inlet Line and Syringe Pump Inlet Line in filtered and degassed water.
- Use PEEK Tubing with same internal diameter as NGC System tubing to connect position 4 on the Autosampler 6-port valve to Loop E (Empty) position on NGC Inject Valve.
- Use PEEK Tubing with same internal diameter as NGC System tubing to connect position 5 on the Autosampler 6-port valve to Loop F (Fill) position on NGC Inject Valve.
- 6. Attach an appropriately sized sample loop to positions 3 and 6 on Autosampler 6-port valve.

**Note:** Positions 1 and 2 on Autosampler 6-port valve should already be connected to the needle (1) and syringe pump (2), respectively.

7. After connecting all ports, take a syringe of equilibration buffer and insert into NGC Inject Valve syringe port, emptying the syringe to purge tubing of air. This volume should be five times the sample loop size.



MVX-7100 Autosampler and 6-port valve.

8. Open the CETAC Workstation Software.

**Note:** If cooling is required, see the instructions in Getting Started — Instrument Setup with Peltier Cooling before proceeding.

- Click the dropdown menu under COM port, and select the appropriate communications port. This depends on where the USB from the autosampler is plugged into the computer.
- 10. Click Initialize Communication.

# **Getting Started — Instrument Setup with Peltier Cooling**

- Prior to Initializing Communication with the Autosampler, click Set Configuration, select Edit under PRM Configuration, then select Yes to indicate the chiller is part of the configuration. Select Save Changes.
- 2. Close the window.
- 3. Click the dropdown menu under COM port, and select the appropriate communications port. This depends on where the USB from the autosampler is plugged into the computer.
- 4. Click Initialize Communication.



- 5. Under the Manual Controls tab, set the temperature in the PRM Controls section and execute by clicking the curved arrow.
- 6. Cooling has begun when the blue light blinks on the Peltier cooling unit. Temperature has been reached when the blue light is solid.

Additional information about instrument setup can be found in bulletin 7194.

## **Getting Started — Priming the CETAC MVX-7100**

After completing Setting Started — Instrument Setup, perform the following steps.

- 1. Click the Manual Controls tab.
- Select 16 from the Pump Address dropdown menu. This is the pump address for the syringe pump, used to load the sample loop.
- 3. Select Fast from the Initialized Speed dropdown menu.
- 4. Initialize the pump by clicking the curved arrow to the right of the dropdown menu for Initialized Speed.
- 5. Prime the pump with water a minimum of six times to remove any air and 20% ethanol storage solution from the system. Do this by typing the number 6 under the Prime Pump selection box, and execute by clicking the circular arrow to the right of the open field box.
  - a. If any air remains in the syringe or syringe line, repeat this step.
  - b. The solution needs to be degassed so that air is not introduced into the lines.
- 6. Click the Autosampler tab.
- 7. Within the pump speed controls, enter **25** and initialize by clicking the curved arrow to the right of the open field window.
- 8. Select **On** from the Pump section to turn the pump on. This is the rinse station peristaltic pump. Allow pump to run for 3–5 min to remove storage solution from the rinse station.
  - a. If you are using the rinse station to chase your sample into the loop, then this buffer must match your equilibration or binding buffer.
  - b. The pump will continue to run until you select Off.
- 9. Place the Rinse Station Pump Inlet Line and Syringe Pump Inlet Line in filtered and degassed equilibration or binding buffer.
- 10. Repeat steps 5-8.

#### **Getting Started — Option 1: Full Loop Pickup Method**

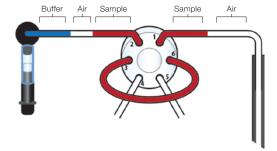
- Open the method in the CETAC Workstation: File > Open > select Full Loop Pickup file. Click Open.
- 2. Double click **FullLoopPickup** in the Methods window on the right side of the screen.
- Double click Select Sample Set (Command 1) under Method Sequence and click Select Tubes to bring up the Tube Selection pop-up window.
  - a. Identify the sample location using the Custom Range window.

- b. Click Make Selection when finished selecting range.
- c. Set the Z-depth. This is based on your sample vessel and instrument configuration settings. In testing, 50 was the depth chosen for 96-well plates. This depth must be independently verified on the user's instrument.
- d. Click **OK**. If the Z-depth calibration has not been completed, a Z-depth error will appear. Click **OK** to bypass the warning. It is recommended to calibrate the Z-depth prior to continuing.
- 4. Double click **Aspirate** (**Command 17**). This Aspirate command is the sample injection volume command and is dictated by the user's experimental parameters. The sample loop should match the desired injection volume.

**Note:** This is the second Aspirate command in this series. The first Aspirate command should not be modified.

- a. Set Syringe Address to 16.
- b. Enter the aspiration volume in Amount field. For Full Loop Pickup, the volume should be **30 µl more than** the sample loop volume. For example, if the experimental setup requires a 100 µl injection, the sample loop on the CETAC MVX-7100 should be 100 µl, and the aspiration volume should be 130 µl. This will result in a full loop fill and the highest reproducibility between samples as there is no diffusion within the sample line.
- c. Set Speed to 2000.000.
- d. Set Valve Position to Output.
- 5. Double click Aspirate (Command 22).
  - a. Set Syringe Address to 16.
  - b. Enter the aspiration volume in Amount field. For Full Loop Pickup, set the volume to  $345~\mu l$ . This volume was optimized for 77 cm length tubing (inner diameter: 0.8 mm) from the injection needle to 6-port injection valve on the CETAC MVX-7100. Any deviation in tubing type or length will need to be reoptimized for this aspiration volume.
  - c. Set Speed to 1000.000.
  - d. Set Valve Position to Output.
- 6. Click Update.
- 7. Click Run Script.

**Note:** The CETAC script must be started prior to starting the NGC System run.



Full Loop Pickup sample loading.

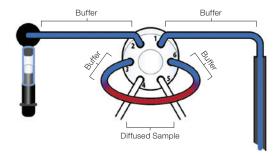
## **Getting Started — Option 2: Partial Loop Pickup Method**

Partial Loop Pickup is a method designed for loading where no sample loss occurs. The CETAC MVX-7100 rinses the needle, picks up the designated amount of sample, moves back to the rinse station, and uses rinse station buffer to move the sample completely into the sample loop. For this method, the sample loop volume on the MVX-7100 must be at least 250  $\mu$ l more than the sample volume size.

- Open the method in the CETAC Workstation: File > Open > select Partial Loop Pickup. Click Open.
- 2. Double click **PartialLoopPickup** in the Methods window on the right side of the screen.
- Double click Select Sample Set (Command 1) under Method Sequence and click Select Tubes to bring up Tube Selection pop-up window.
  - a. Identify the sample location using the Custom Range window.
  - b. Click Make Selection when finished selecting range.
  - c. Set the Z-depth. This is based on your sample vessel and instrument configuration settings. In testing, 50 was the depth chosen for 96-well plates. This depth must be independently verified on the user's instrument.
  - d. Click **OK**. If the Z-depth calibration has not been completed, a Z-depth error will appear. Click **OK** to bypass the warning.
     It is recommended to calibrate the Z-depth prior to continuing.
- 4. Double click Aspirate (Command 15).
  - a. Set Syringe Address to 16.
  - b. Enter the aspiration volume in Amount field. For Partial Loop Pickup, the aspiration volume should equal the sample volume. For example, if the experimental setup requires a 100 µl injection, the sample loop volume on the CETAC MVX-7100 should be at least 350 µl and the aspiration volume should be 100 µl. This will result in no sample loss as the entire sample, sandwiched by buffer, is moved to the sample loop.
  - c. Set Speed to 2000.000.
  - d. Set Valve Position to Output.
- 5. Double click Aspirate (Command 24).
  - a. Set Syringe Address to 16.
  - b. Enter **500**  $\mu$ I for the aspiration volume in Amount field. For Partial Loop Pickup, setting this volume moves the sample from the needle and into the sample loop with excess chase volume to ensure the entire sample is retained in the sample loop.
  - c. Set Speed to 5000.000.
  - d. Set Valve Position to Output.
- 6. Click Update.
- 7. Click Run Script.

**Note:** The CETAC script must be started prior to starting the NGC System run.

If you have multiple volumes, use the Partial Loop Pickup Series file.



Partial Loop Pickup sample loading.

## **Getting Started — Option 3: Partial Loop Pickup Series Method**

Partial Loop Pickup Series is a set of methods that allows you to change the volume between sample sets. Similar to Partial Loop Pickup, these methods chase the sample with excess buffer to the sample loop.

- 1. Open the method in the CETAC Workstation: File > Open > select **Partial Loop Pickup Series**. Click **Open**.
- For the first sample volume, double click
   PartialLoopPickupVolume1 in the Methods window on the right side of the screen.
- Double click Select Sample Set (Command 1) under Method Sequence and click Select Tubes to bring up Tube Selection pop-up window.
  - a. Identify the sample locations using the Custom Range window, choosing samples that correspond to sample volume 1.
  - b. Click Make Selection when finished selecting range.
  - c. Set the Z-depth. This is based on your sample vessel and instrument configuration settings. In testing, 50 was the depth chosen for 96-well plates. This depth must be independently verified on the user's instrument.
  - d. Click **OK**. If the Z-depth calibration has not been completed,
     a Z-depth error will appear. Click **OK** to bypass the warning.
     It is recommended to calibrate the Z-depth prior to continuing.
- 4. Double click Aspirate (Command 15).
  - a. Set Syringe Address to 16.
  - b. Enter the aspiration volume in Amount field. For Partial Loop Pickup, the aspiration volume should equal the sample volume. For example, if the experimental setup requires a 100  $\mu$ l injection, the sample loop volume on the CETAC MVX-7100 should be at least 350  $\mu$ l and the aspiration volume should be 100  $\mu$ l. This will result in no sample loss as the entire sample, sandwiched by buffer, is moved to the sample loop.
  - c. Set Speed to 2000.000.
  - d. Set Valve Position to Output.

- 5. Double click Aspirate (Command 24).
  - a. Set Syringe Address to 16.
  - b. Enter **500** µI for the aspiration volume in Amount field. For Partial Loop Pickup, setting this volume moves the sample from the needle and into the sample loop with excess chase volume to ensure the entire sample is retained in the sample loop.
  - c. Set Speed to 5000.000.
  - d. Set Valve Position to Output.
- For the second sample volume, double click
   PartialLoopPickupVolume2 in the Methods window on the right side of the screen.
- Double click Select Sample Set (Command 1) under Method Sequence and click Select Tubes to bring up Tube Selection pop-up window.
  - a. Identify the sample locations using the Custom Range window, choosing samples that correspond to sample volume 2.
  - b. Click Make Selection when finished selecting range.
  - c. Set the Z-depth. This is based on your sample vessel and instrument configuration settings. In testing, 50 was the depth chosen for 96-well plates. This depth must be independently verified on the user's instrument.
  - d. Click **OK**. If the Z-depth calibration has not been completed, a Z-depth error will appear. Click **OK** to bypass the warning.
     It is recommended to calibrate the Z-depth prior to continuing.
- 8. Double click Aspirate (Command 15).
  - a. Set Syringe Address to 16.
  - b. Enter the aspiration volume in Amount field. For Partial Loop Pickup, the aspiration volume should equal the sample volume.
  - c. Set Speed to 2000.000.
  - d. Set Valve Position to Output.
- 9. Double click Aspirate (Command 24).
  - a. Set Syringe Address to 16.
  - b. Enter  $500~\mu l$  for the aspiration volume in Amount field.
  - c. Set Speed to 5000.000.
  - d. Set Valve Position to Output.
- 10. Repeat steps 2–5 for additional sample volumes using PartialLoopPickupVolume3-6 in the Methods window on the right side of the screen.

**Note:** If you have fewer than six different injection volumes, remove any methods not needed by highlighting the additional volume methods and clicking **Remove**.

11. Click **Update**. Then click **Run Script**.

**Note:** The CETAC script must be started prior to starting the NGC System run.

#### **Shutting Down the CETAC MVX-7100 Autosampler**

- 1. Place the Rinse Station Pump Inlet Line and Syringe Pump Inlet Line in filtered and degassed water.
- 2. In the Manual Control tab, initialize the pump by clicking the curved arrow to the right of the dropdown menu for Initialized Speed.
- 3. Prime the pump with water a minimum of six times to remove any buffer from the system. Do this by typing the number 6 under the Prime Pump selection box, and execute by clicking the circular arrow to the right of the open field box.
- 4. Click the Autosampler tab.
- 5. Within the pump speed controls, enter **25** and initialize by clicking the curved arrow to the right of the open field window.
- Select **On** from the Pump section to turn the pump on. This is the rinse station peristaltic pump. Allow pump to run 3–5 min to remove buffer from the rinse station. The pump will continue to run until you select **Off**.
- 7. Place the Rinse Station Pump Inlet Line and Syringe Pump Inlet Line in 20% ethanol or another bacteriostatic solution.
- 8. Repeat steps 3-6.
- 9. Turn instrument off and shut down software.

# **Ordering Information**

Catalog # Description

#### **Bio-Rad Laboratories**

7880001 NGC Chromatography System with ChromLab Software,

version 4.0 or higher

7884016 NGC System Signal Import Module

#### **Additional Tools**

Use Teledyne CETAC MVX-7100 Bio-Rad Configuration (part #A71-99-0016HR) or Teledyne CETAC MVX-7100 Bio-Rad Configuration with Temperature Control Block (part #A71-99-0015HR).

Visit bio-rad.com/NGC-MVX7100 for more information.

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