K&A LABS GMBH

K&A Oligo Synthesizer

Owner's Manual

Introduction

Overview

About This Chapter This chapter provides an overview of the K&A Oligo Synthesizer documentation, safety considerations, and technical support resources available.

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K&A Synthesizer Documentation

Background Needed This manual assumes that you are familiar with the following:

- Basic Windows OS operations, such as using the mouse, selecting commands, working with windows, and using the Windows OS computer file management system
- The general manipulation of data files
- Good laboratory practices and basic laboratory techniques
- Oligo synthesis chemistry

About the Documentation Set This three-quarter view shows you the front and the left side of the K&A instrument. Use the following table to determine which K&A instrument document you need for the task at hand. All of the documents listed are sent to K&A instrument customers.

If you want...	Refer to the
• To prepare your laboratory for installation of the instrument • The instrument's electrical, ventilation and space requirements • A Site Preparation Checklist • Explanations of instrument safety alert symbols in several languages	Preinstallation Guide
• Instructions for general instrument setup and run initiation using pre-programmed cycles • Routine maintenance information • Operational safety information	User's Manual

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Sierra BioSystems user documentation. Each word implies a particular level of observation or action as described below.

Note – Calls attention to useful information.

IMPORTANT! – Indicates information that is necessary for proper instrument operation.

DANGER! - Indicates if the danger is not avoided, it will cause death or serious injury.

WARNING! - Indicates if the warning is not heeded, it can cause death or serious injury.

CAUTION! - Indicates if the precaution is not taken, it may cause minor or moderate injury.

Chemical Hazard Warning

WARNING! Chemical Hazard. Some of the chemicals used by the K&A instrumentation and protocols are potentially flammable.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous material.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or protective clothing).
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If leaks or spills occur, follow the manufacturer's cleanup procedures as recommended by the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

WARNING! Chemical Hazard. The K&A ships with default protocols for oligonucleotide production. Within these protocols, flammable liquids are set to dispense in volumes smaller than 50µl. Alteration of these protocols is not recommended by Sierra BioSystems, Inc.

Instrument Safety Labels

Safety Labels are located on the instrument. Each safety label has three parts:

- A signal word panel, which implies a particular level of observation or action (e.g., CAUTION or WARNING). If a safety label encompasses multiple hazards, the signal word corresponding to the greatest hazard is used.
- A message panel, which explains the hazard and any user action is required.
- A safety alert symbol, which indicates a potential personal safety hazard.

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Read and understand all related MSDSs.

CAUTION! Avoid using this instrument in a manner not specified by Sierra BioSystems, Inc. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.

14		ISO 7000 - 0434B (2004-01)	Caution *

Laboratory Environmental Requirements

Altitude	This instrument is for indoor use only and for altitudes not exceeding 2000 m (6500 ft) above sea level.
Temperature and Humidity	Laboratory temperature should be between 16-22°C (60-72°F), but the instrument can handle temperatures of between 10°C and 40°C. The instrument can tolerate maximum relative humidity of 99%.
Pollution	This instrument may be installed in an environment with nonconductive pollutants only.

Electrical Requirements

Location	Voltage (VAC)	Frequency	Amperage (A)
USA/Canada	120 ± 10%	50/60 Hz ± 1%	1
Europe	240 ± 10%	50/60 Hz ± 1%	1
Australia	240 ± 10%	50/60 Hz ± 1%	1
Japan	110 ± 10%	50/60 Hz ± 1%	1

Power Line The electrical receptacle must have a dedicated 1.5 kVA power line and ground or a 1.5 kVA power line with a line conditioner or uninterruptible power supply (UPS).

Power Rating This instrument is rated for a maximum output of 240 W

Power Cords In the USA, Canada, and Japan, the instrument is supplied with a detachable cord equipped with a standard three-prong plug.

In Europe and Australia, the instrument is supplied with a detachable electrical cord equipped with a standard EC plug.

Grounding Certain types of electrical noise are greatly exaggerated by poor or improper electrical ground connections. To prevent these problems, it is very important to have a dedicated line and ground between the instrument and building main electrical service.

Placing the Instrument

Note: Please refer to the K&A Preinstallation Guide for a thorough explanation of all site preparation and preinstallation procedures.

Guidelines for Lifting and Carrying the K&A

WARNING! Movement of the instrument should never be attempted without first being purged and cleaned of all reagents.

Using a lab bench with a minimum carrying capacity of 200 lbs, center the K&A over the table in both width and depth. The weight of the K&A is evenly distributed across the machine, so a direct, vertical lift with evenly balanced force is advised.

Once the K&A has been located, place the instrument so that all four corners are seated flat onto a secure surface.

Installing the Instrument

The K&A ships with all necessary components for dry operation. This includes:

- The K&A Synthesizer
- Computer with Windows OS and K&A Software
- Power Cords (4)
- Ethernet Cord
- Computer Monitor and Keyboard/Mouse
- Air Compressor

Using these components, following the steps below to install the instrument:

1. Locate the K&A onto a sturdy table following the guidelines described in “Placing the Instrument”.
2. Place the computer either beside or underneath the K&A, and attach the provided monitor, keyboard, and mouse to the computer.
3. Place the monitor, keyboard, and mouse in a viewable/reachable area.
4. Attach the provided Ethernet cord to the Ethernet port on the K&A and to any Ethernet port on the computer. Be sure the Ethernet cord sounds an audible “click” as it affixes to the port.
5. Plug the given power cord into a nearby socket and then the back of the instrument. Be sure the socket used provides a grounding source.

Uninstalling the Instrument

When uninstalling the K&A, the user will must observe all procedures listed in Long-Term Shutdown, and be sure to account for the following:

- The K&A Synthesizer
- Computer with Windows OS and K&A Software
- Power Cords (4)

- Ethernet Cord
- Computer Monitor and Keyboard/Mouse
- Air Compressor

1. Begin by following the steps listed in Long-Term Shutdown
2. Power-off the instrument using the primary power switch located on the back of the instrument.
3. Power-off the computer after saving all necessary data.
4. Disconnect the power cord first from the machine and then from the outlet.
5. Disconnect the ethernet cord first from the machine and then from the computer.
6. Disconnect the computer monitor, keyboard, and mouse.
7. Recollect and bag all cords and computer paraphernalia.

For any questions or concerns, please contact:

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Tour of the Instrument

Overview

About This Chapter This chapter provides an overview of the K&A Oligo Synthesizer hardware and the software components that you will use most often.

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About the K&A

Overview

The K&A Synthesizer couples single nucleotides (bases) together in a stepwise fashion to form customized oligonucleotides linked to a solid support.

Engineered for maximum flexibility, the K&A features advanced software capabilities that allow for the synthesis of heavily modified oligonucleotides, including novel nucleotide incorporations and phosphate backbone modifications. This flexibility makes it well-suited for research applications requiring customized synthesis protocols beyond standard oligonucleotide production.

Software

The K&A software is installed on the system computer. Use the K&A software to perform:

- Synthesis runs
- Protocol design
- Calibration functions
- Manual Control functions
- Priming functions
- Maintenance and Diagnostic operations

How the K&A System Works

The K&A is equipped with **amidite positions, reagent positions, and positions for synthesis columns**. Amidite and reagent positions can be assigned interchangeably depending on synthetic requirements, providing flexibility in synthesis design.

The system operates entirely on **argon gas pressure**, with no internal moving parts such as pumps, motors, or electric valves—only gas-actuated valves control fluid flow.

All fluid connections are **easily accessible on the front panel**, and consumables such as cartridges, reagents, and bottles are readily available from multiple vendors.

The system's versatile software allows **precise control over amidite handling, reagent selection, and reaction sequencing**, giving chemists the freedom to design **custom synthetic routes, incorporate unusual reagents, and produce heavily modified oligonucleotide sequences**.

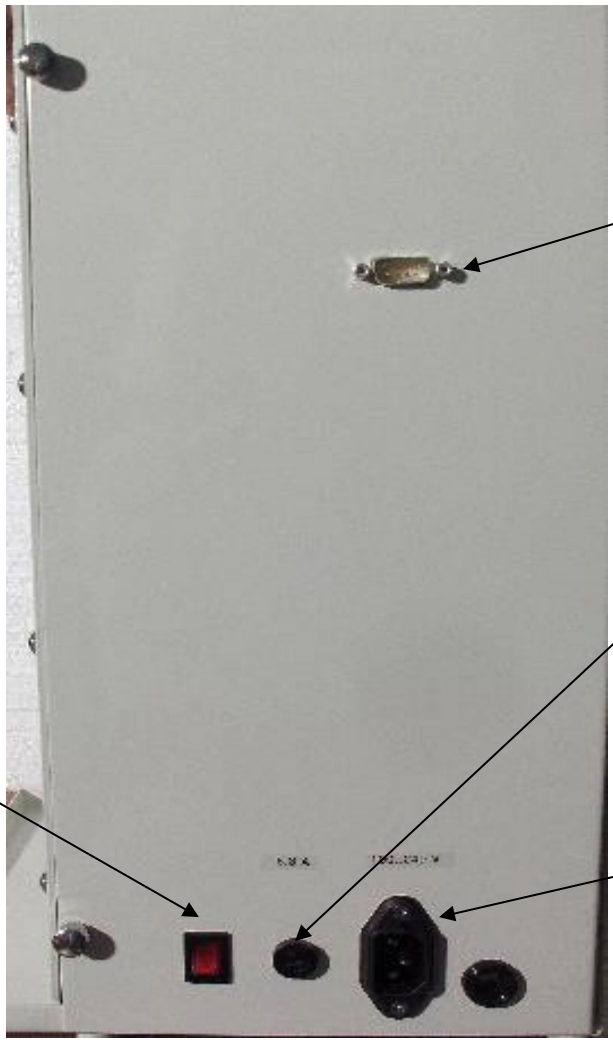
Unlike traditional oligonucleotide synthesizers, which are often limited to standard sequences, this system enables a broader range of synthesis applications.

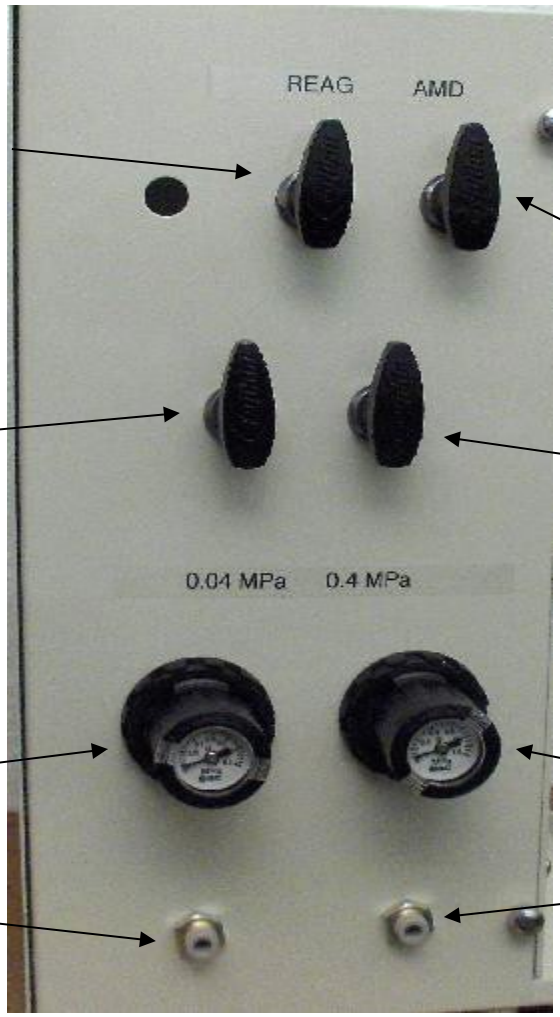
Synthesis Protocols

A Synthesis Protocol tells the instrument the steps to perform in order to synthesize the DNA sequences you have entered. An optimal cycle program is provided with the K&A instrument software and is the only type of oligonucleotide production that will be covered in this manual. However, you can also program cycles to further customize the DNA synthesis process in your laboratory.

The K&A Synthesizer's closed-column modality offers a high degree of freedom in the creation of synthesis protocols and oligo handling, allowing the user to run synthesis scales beyond that of traditional synthesizers and handle the oligo with ease after the completion of the synthesis.

K&A Synthesizer Hardware


Connect cable
to computer
Fuse 3.15 A
Main power
switch
Power cord



Argon pressure for reagent bottles excl. activator bottle

Argon pressure for amidite bottles incl. activator bottle

Argon tap

Compressed air tap

Gauge and manometer for argon 0.04 MPa

Gauge and manometer for pressed air 0.4 MPa

Argon inlet

0.4 MPa

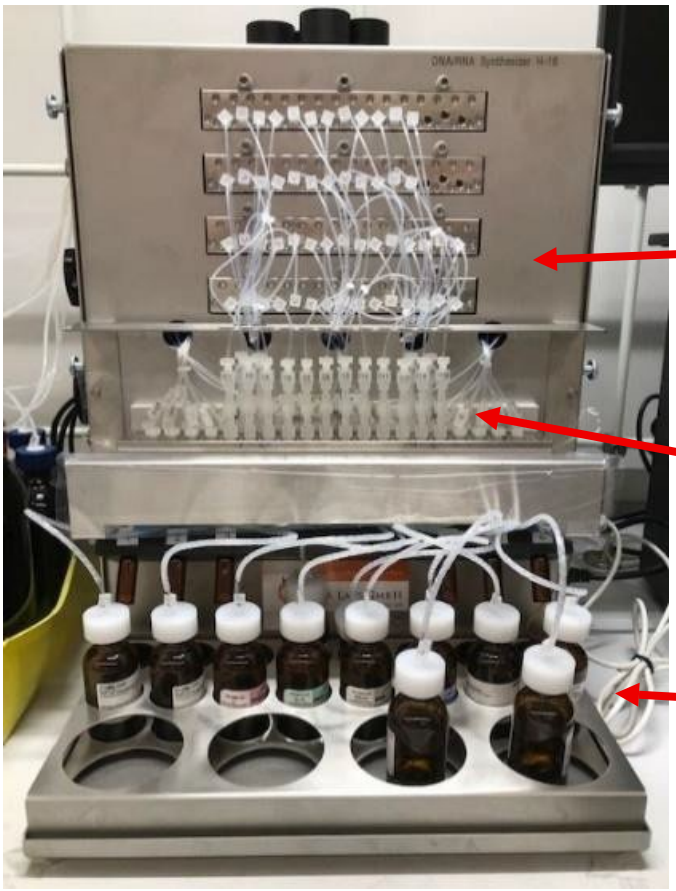
Pressed air inlet

0.4 MPa

Synthesizer Installed (example)

Waste and Air Compressor below the synthesizer

Synthesizer Installed (continued)



NOTE: Each amidite bottle has its own single-letter label for identification. There are specific bottles for natural A, G, T, and C and additional bottles (such as E and F in the picture above) for special amidites.

Reagent bottles are also labeled with identifier tags that correspond to the nomenclature of these bottles in the software. For example, “OXI” refers to the oxidizer (such as iodine in THF, pyridine, and water) both on the bottle label tag and in the software abbreviation for this reagent.

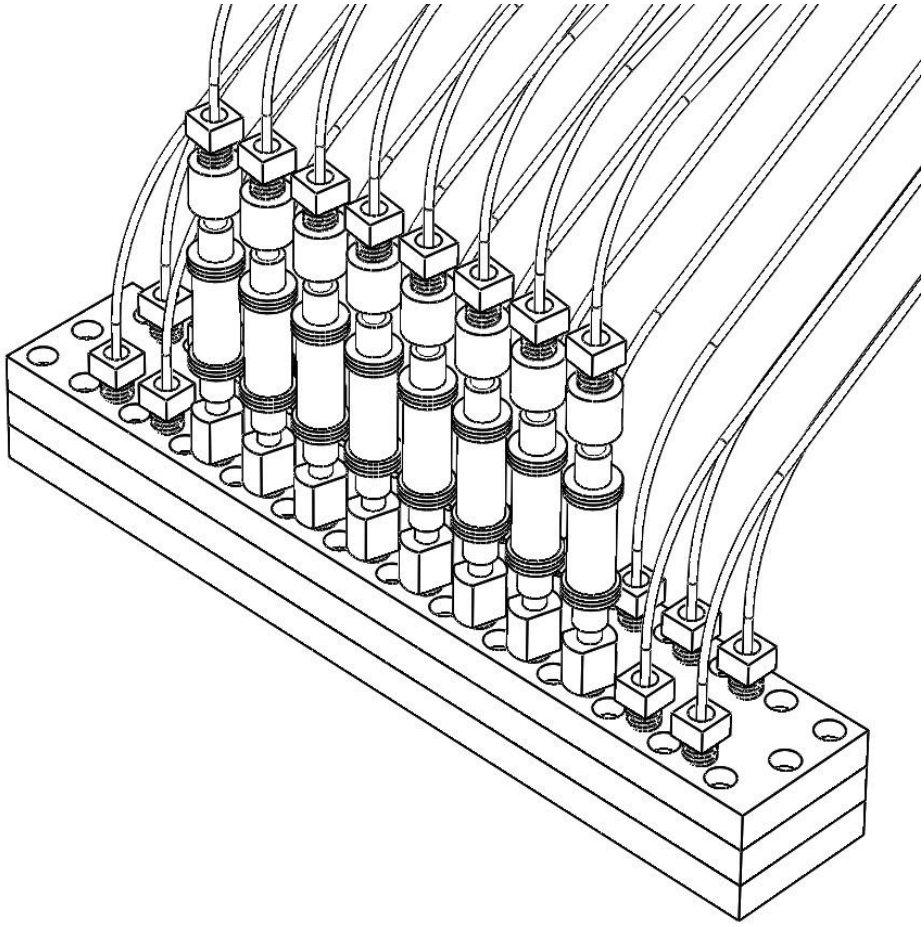
Column Close-Up:

Bypass Connector vs Synthesis Column:

The Membrane

Membrane Operating Principles

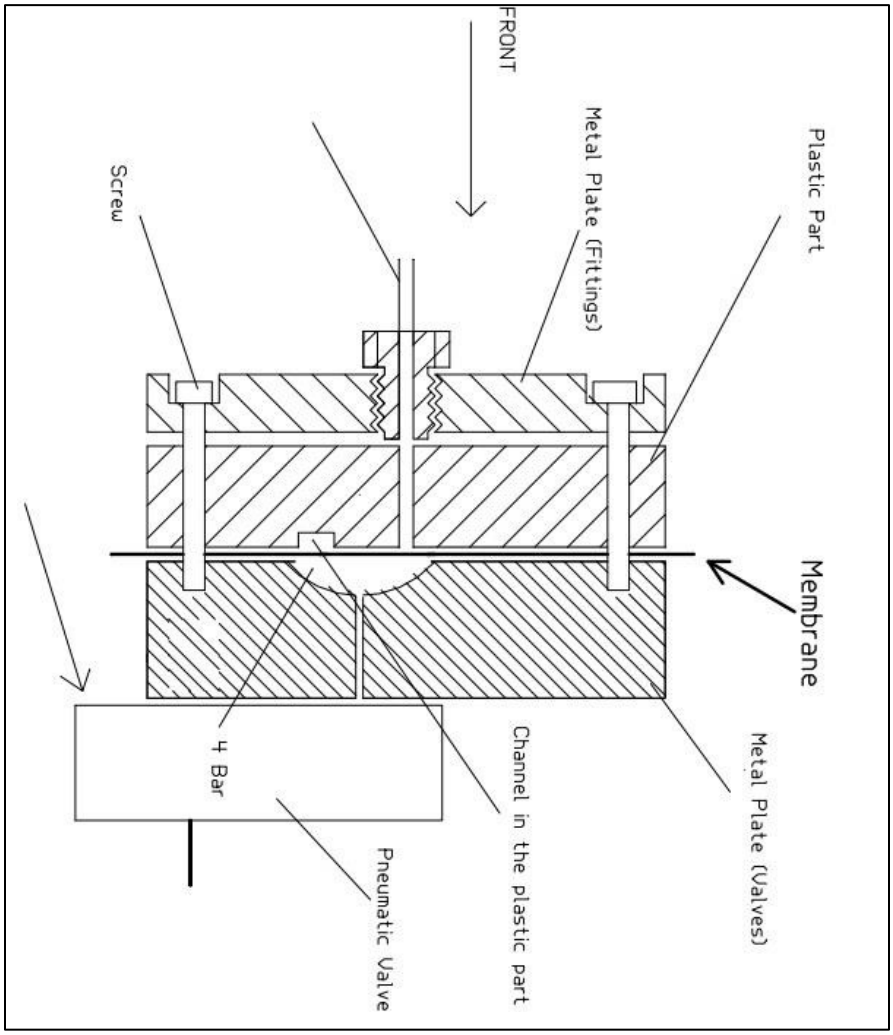
The K&A system manages fluid movement through a common manifold, pneumatic valves, and a single sealing membrane. This design ensures controlled fluid delivery by regulating pressure and valve actuation.



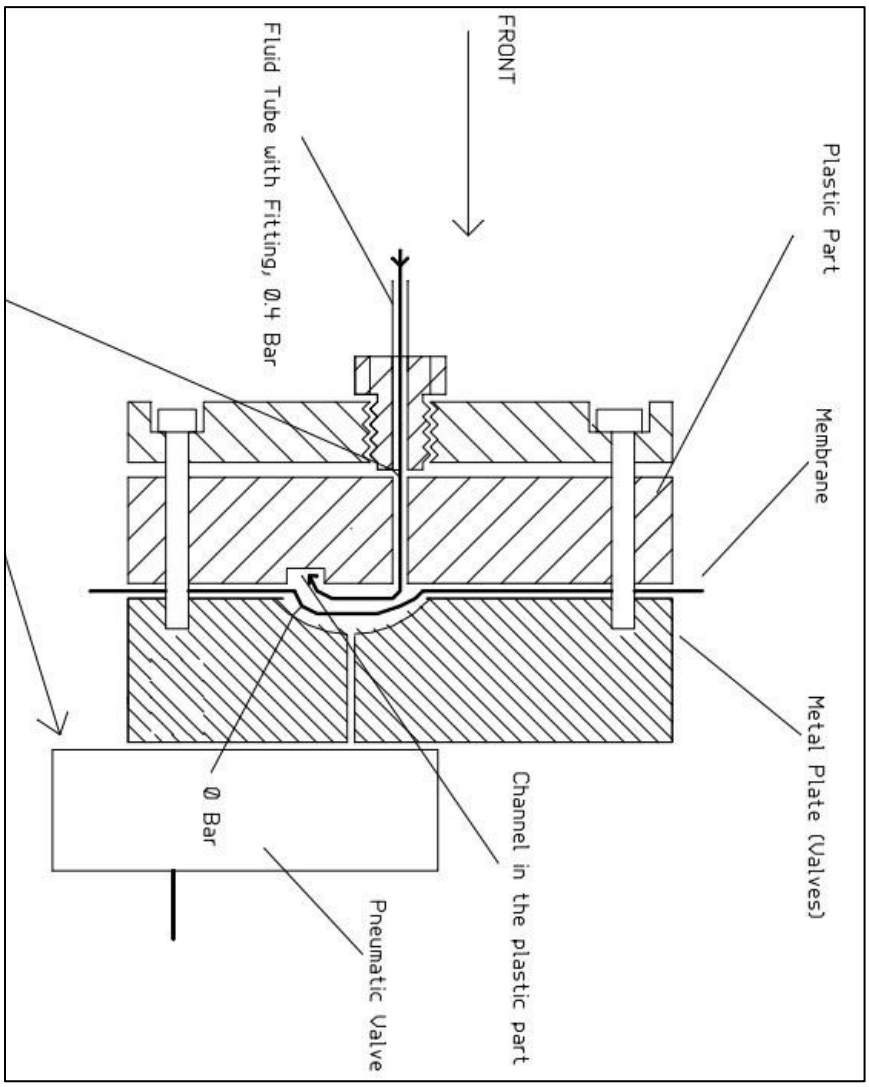
Fluids are stored in pressurized bottles at 0.04 MPa and flow toward the sealing membrane. On the opposite side of the membrane, compressed air at 0.4 MPa is applied through dedicated pneumatic valves.

This air pressure holds the membrane tightly against the fluidic connections, preventing fluid from passing into the manifold.

Membrane Sealed



Membrane Relieved



When a pneumatic valve opens, compressed air is vented to the atmosphere. With the counterpressure removed, the fluid at 0.04 MPa pushes against the membrane and flows into the common manifold channel. The system continuously cycles through valve actuations as needed, briefly releasing the membrane to allow fluid transfer before resealing.

By maintaining precise pressure control, the K&A system ensures reliable fluid handling, preventing leaks and unintended flow.

About the Software

Overview

The K&A Synthesizer software graphical user interface allows you to enter information about the sequences you are synthesizing and to operate the instrument. The GUI allows you to easily:

- Enter custom sequences in several ways:
  - Individually, by typing or cutting and pasting from a text file
  - Importing single sequences via .txt, .csv, or .xlsx files
  - Importing entire lists of sequences via .txt, .csv, or .xlsx files
- Use the optimized protocols that are included in the software, or customize the cycles using the GUI
- Save all sequence and protocol information to repeat the run without re-entry

Conventions Used

Icon directed submenus are available through the Main Toolbar. Each activity operable in isolation will be accessed through its respective submenu.

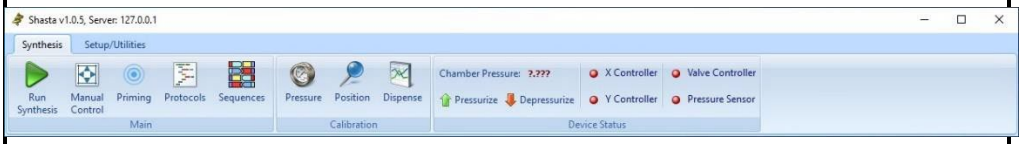
Cascading menus and other Microsoft® Windows operating procedures/commands are not used in this software.

Desktop Icon and Main Toolbar

The K&A Software will be accessible from the Desktop or Windows Explorer software via the K&A icon shown here:

KA_Labs.exe

The Main Toolbar will appear as follows:



From the Main Toolbar, there are two Menus: Synthesis and Setup/Utilities. Each menu contains icons that direct the user to a synthesizer action.

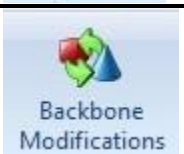
Synthesis tab icons:

	**Run Synthesis** Allows the user to run syntheses by joining protocol and sequence files together. Other actions such as Pause, Stop, Estimate, and View Plate are available here.
	**Manual Control** Gives the user access to all valves on the instrument as well as control over the positions system. Manual Dispense, Pulse. And Drain commands are available here.

	**Priming** A tool used to prime each delivery tip before the synthesis.
	**Protocols** The K&A's proprietary protocol writer. Standard loops as well as Meta-Protocol builders are available here.
	**Sequences** The K&A's proprietary sequence writer. Export and Import functions to and from Microsoft® Excel are available here.
	**Fluid Calibration** Dispense valve calibration utility

The Setup/Utilities tab will appear as follows:

Setup/Utilities icons:

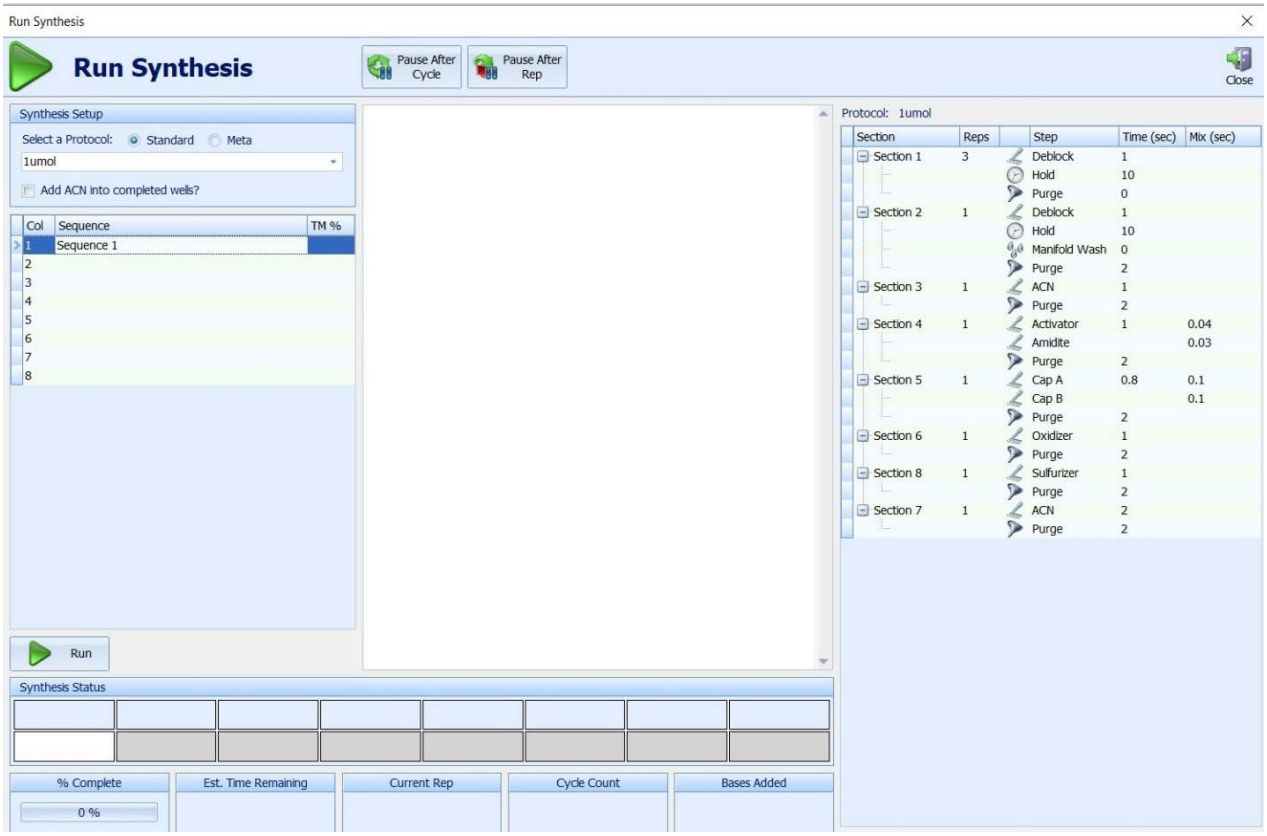
	**Configuration** Window for assigning global variables such as set bands, array patterns, sensor engagement, mid synthesis priming, and other functions.
	**Reagents/Amidites** Stores all information related to plumbed fluids. Identity, density, and other necessary values can be input and retrieved from here.
	**Valve Tip Assignment** Assigns each valve a place on the Valve Controller board.
	**Dispense Tip Assignments** Assigns each valve nozzle a location on the dispense array.
	**Backbone Modifications** Create callouts for backbone modifications in this window, e.g. "Thioate".

	**Manage Servers** Directs the K&A client to which server will be delivering database information.
	**Backup/Restore** Allows the user to backup the existing K&A database or load a new K&A database.

Software Walkthrough

Below is a cursory overview of each screen that will be regularly employed by the user. A more thorough description of key software elements will be detailed in other parts of this manual.

Run Synthesis



The **Run Synthesis** window is the primary interface from which users will operate the instrument. This window will allow the pairing of Sequence and Protocol files to generate a synthesis run.

The **Run Synthesis** window offers additional tools and features to supplement the synthesis:

- **Pause After Cycle** – Sets a pause point for the end of the current cycle. The synthesis will remain paused until the Resume function is selected by the user.
- **Pause After Rep** – Sets a pause point for the end of the current rep of the current section. The synthesis will remain paused until the Resume function is selected by the user.
- **Select a Sequence –** All sequences generated via the Sequence Writer will be available for selection here.
- **Select a Protocol –** All protocols generated via the Protocol Writer will be available for selection here.
    - **Standard** – Standard protocols will run a single protocol for all columns and all bases on the plate.
    - **Meta** – Meta protocols are a combination of protocols specified by the user that allow base-specific, column-specific, and sequence-step-specific handling. This will also join Pre-Process, Post-Process, and DMT-off protocols into one cohesive synthesis.

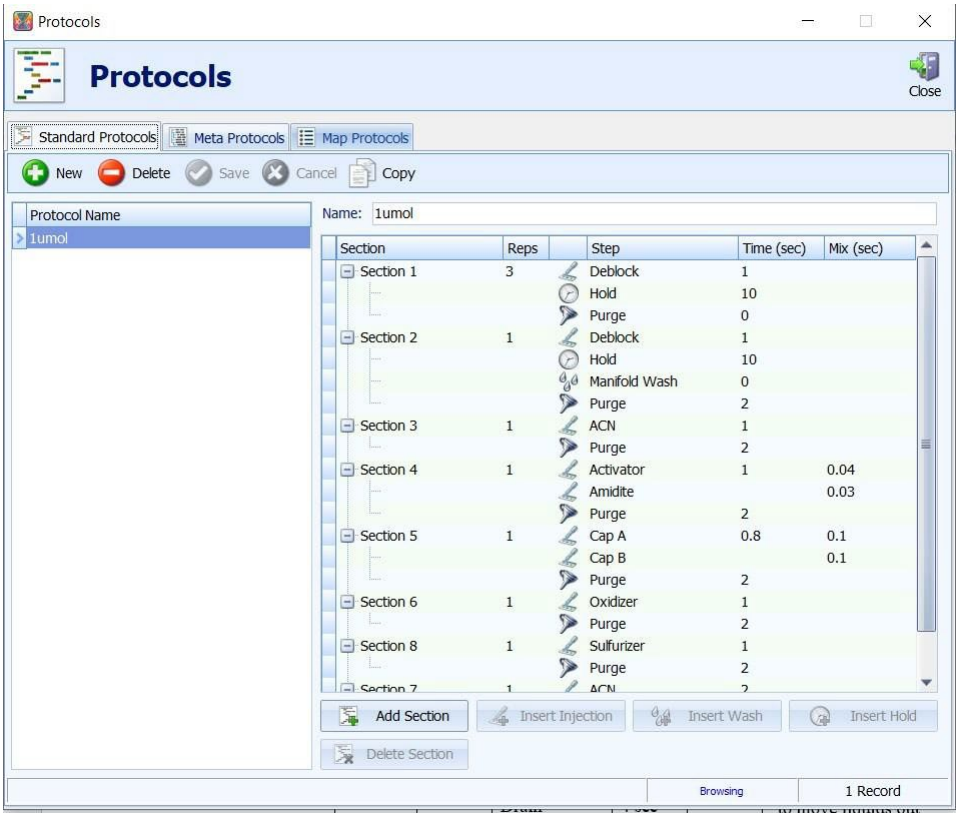
- **Color Display –** This display will show the amidites being handled by the current cycle. The display will also show the column types required by the loaded Sequence file. **IMPORTANT!** Users can terminate a sequence mid-run by *Ctrl + Left Clicking* a well in the Color Display.
- **Protocol Table** – Shows a live feed of the current cycle and the current synthesis step within the file.
- **TM % --** Shows the current estimated yield of the oligo via trityl waste (soon to be updated)

Sequences

Sequences are saved into the K&A database via the **Sequences** window. All sequences must be written, imported, or copied into this window before they can be run in a synthesis.

- **Name** – Allows the user to call out the sequence by a unique name. This will be displayed in the software, log, and exported .xlsx file.
- **Oligo ID –** Functionally the same as "Name" and will likely be omitted in a future update.
- **DMT Off** – Employs the DMT Off protocol within the Meta Protocol when selected.
- **Univ** – Indicates the usage of a universal support. If deselected, the 3' base in the sequence will be skipped as this implies that base is already coupled to the support.
- **Pre** – Employs the Pre protocol within the Meta Protocol when selected.
- **Post** – Employs the Post protocol within the Meta Protocol when selected.
- **Sequence 5' – 3' –** The sequence as it is to be synthesized. These conventions must be followed:
    - All single character or digit nmaes can be listed sequentially, without interuption.
    - Multiple digit or character names must be bracketed, e.g. [11] or [kA]
    - Backbone punctuation must be included
    - There is no case sensitivity. "A" and "a" will be treated the same.
- **Import/Export** – The software can import .xlsx or .csv files. Export a test sequence first as an example. That will demonstrate which cells should contain which of the above options and how they are to be input.
- **Copy** – Allows the user to make a copy of any sequence.

Standard Protocols



The **Protocol Writer** is a tool that allows users to write and edit synthesis protocols. Protocols are seperated into Standard, Meta, and Map protocol files.

The **Standard Protocol** writer is the primary protocol editor. From here, individual cycle files can be written and then applied to sequences, columns, bases, drain groups, etc. There are a few operators available to the user when writing a Standard protocol.

- **Add Section / Delete Section** – Allows the user to implement or remove a section. Sections are discrete routines within the cycle that typically consist of one dipense step followed by a series of actions designed to facilitate the use of that dispense. **NOTE:** Section names are labels only and not used by the software in any way. Section names are defined by the user and intended to mark the beginning of its section.
- **Insert Dispense / Delete Dispense –** Adds or removes a dispense step. Once implemented, dispense steps will be defined with the Dispense drop-down and units in the Time column. The Time is in seconds. Fractional seconds are allowed up to 2 decimal points.
- **Insert Hold / Delete Hold** – Adds or removes a Hold step. Hold steps are waiting periods that have no action other than to allow the reaction on the support to occur.
- **Purge** – Purge steps are timed commands sent to the drain valves. The function of the Purge command is to move liquids out of the column with gas. NOTE: in the H-8-VLS and H-4 models, clicking **Purge** will

convert the action to **Rev Purge** which pushes the fluids out through the bottom of the column rather than the top.

- **Wash** – This is a Manifold Wash step that sends ACN through the manifold and is follwed by Gas. The Time listed for this will apply to both ACN and GAS, i.e. a "2" in the Time column will send 2 seconds of ACN followed by 2 seconds of Gas.
- **Mix –** The Mix column is used when two Dispenses are listed sequentially in the same section. When utilized, power to the valves will oscillate between the desired ports to create a "mix" of the desired solutions on their way to the column. The user will input the "valve open" time for each valve in the mix, and they will oscillate in this pattern until the value in the "Time" column is reached. For example:

Section	Reps	Step	Time	Mix
Couple	1	Activator	2.5	.04
		Amidite		.03
		Hold	60 sec	
		Drain	4 sec	

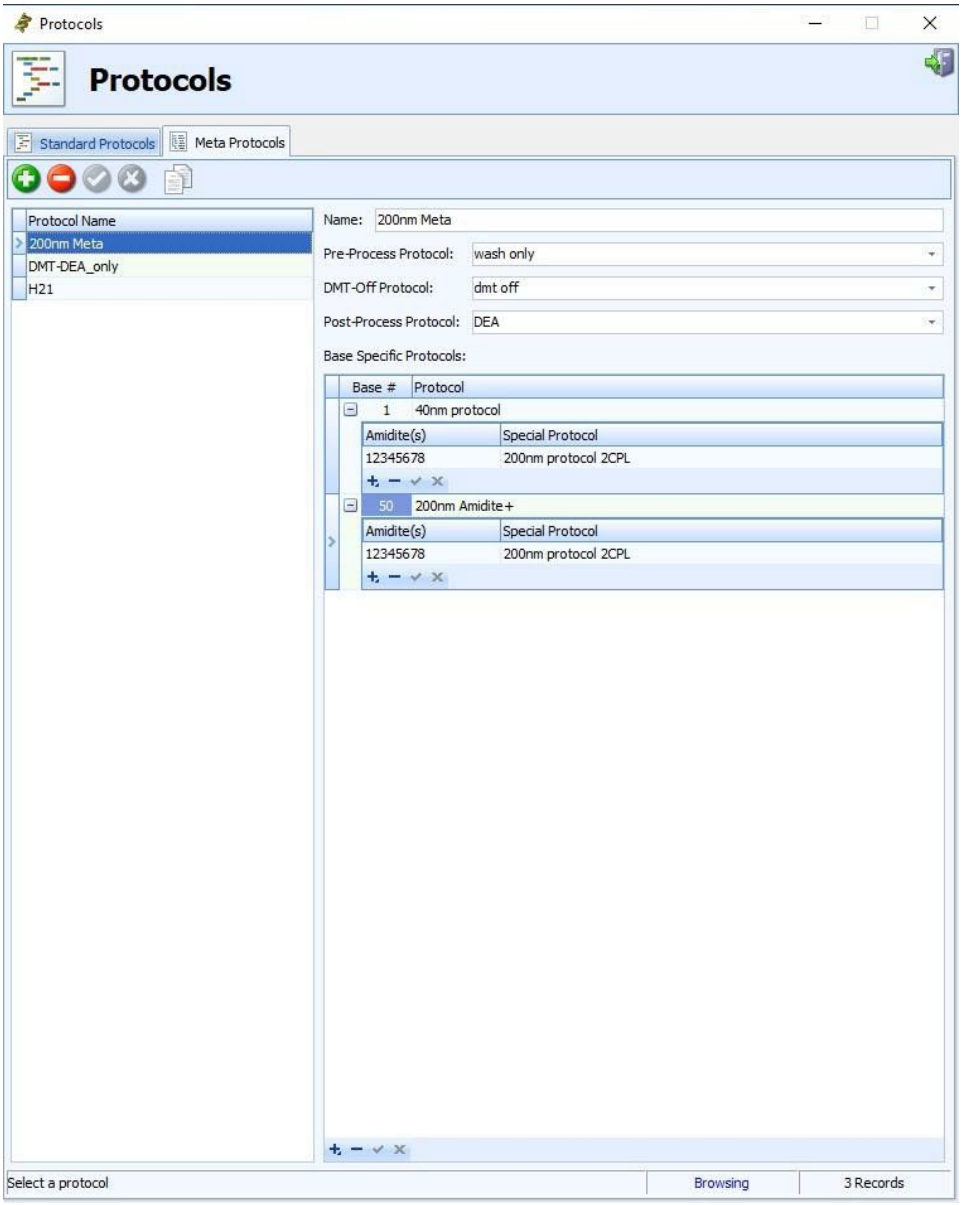
This coupling step will inject Activator for .04 seconds, follwed by an Amidite for .03 seconds until a total time of 2.5 seconds has been reached.

NOTE: up to 8 positions can be mixed at a time. This includes all reagents. For mixed base couplings, please use the Mixed Base function in the Reagent/Amidite assignemnt window.
NOTE: the input TIME only needs to be typed in the first dispense's time slot. Inserting a time value in the subsequent slots will have no effect.

Zero Values are Allowed – If a zero value is input, it is as if the step is skipped entirely. This may be useful if the user wants to:

1) Skip the purge step. If there are liquids in the column that need to remain during the next injection.
2) Skip injections for certain bases within a bank. If only one base requires a unique reagent injection, but it is within a bank of sequences that should not receive this reagent, then typing a "0" for all of the "skipped" bases, and typing the desired volume for the desired base, will keep the injection isolated to the one, desired base.

Meta Protocols



The **Meta Protocol** writer is the secondary protocol editor. From here, Standard Protocols can be linked together to automatically perform case-dependant operations. Base handling, Pre- and Post-synthesis procedures, DMT removal, and bulk synthesis changes throughout the run can be effected here.

- **Pre-Process Protocol** – The Standard Protocol placed in this slot will run before the synthesis begins. No bases will be coupled in this step. It is inteded for a pre-synthesis wash, extra Deblock to remove less labile linker, etc.
- **DMT-Off** – The Standard Protocol placed in this slot will be run immediately after the synthesis has completed. It is intended as a final DMT removal step, but any protocol that does not require phosphoramidite could be performed here. **IMPORTANT!** The

synthesizer will not automatically remove the final DMT group unless specified in both this slot and in the sequence file.

- **Post-Process Protocol** – The Standard Protocol placed in this slot will be run immediately after the DMT-Off step, and it will be the final protocol in the synthesis. This cycle is intended for DEA treaments, additional DCM rinses, etc.
- **Base-Specific Protocols –** The Base-Specific Protocol drop-downs are where the primary synthesis cycles will be defined. Which phosphoramidites will receive particular treatments and *when* they are to receive them will be decided here.
    - **Base #** -- This column defined when certain protocols will be engaged over the course of the synthesis. By default, the first row in the Base-Specific Protocol window will be "1" because we are engaging the decided protocol on Base 1, i.e. the start of the synthesis. **NOTE:** This does not mean that specialty phosphormaidite 1 will use this protocol. It is a time stamp only...
      Additional protocols can be engaged throughout the synthesis by selecting the "+" at the bottom of the window. Assigning this new row's Base # will decided when in the sequence this change is to be made. For example, Base # "20" will begin using the specified protocols at cycle 20 of the synthesis.
    - **Protocol** – To the right of Base # is the Protocol column. This is where the protocol for every base (unless specified otherwise in Special Protocols) will be decided. **IMPORTANT!** This slot cannot be empty. It is the primary protocol to be run during the synthesis.
    - **Amidite(s)** – This is where phosphoramidites that will require special handling can be selected. The column can have many rows, and each row will contain phophoramidites with unique handling requirements. Phosphoramidites that share handling requirements will likely share a protocol, and thus, share a row in this column. **NOTE:** Base names exceeding a single character must be enclosed by "[ ]", e.g. base "FAM" would be inserted as [FAM].
    - **Special Protocol** – The protocol inserted into this space will take effect for each base listed to the left in the Amidite(s) column. **IMPORTANT!** The entire protocol can be changed for each amidite, but because special and standard phophoramidites will often exist in the same drain group, it is important that section and dispense order be the same for special and standard protocols. Injetion volumes, holds, pulses, and drain times can all vary.

Special Base Handling

The K&A allows the user to modify every step of the protocol for every base. This is done by inserting "sub-amidite groups" underneath the primary protocol.

For example:

The primary protocol in this picture is “40nm protocol”. It will be applied to every base that is not in the sub-amidite group.

The sub-amidite groups consisits of bases labeled 1, 2, 3, 4, 5, 6, 7, and 8. These bases will follow another protocol called “200nm protocol 2CPL”. So, in this example, because A, C, G, etc. are not listed in the sub-amidite group, they will follow the primary protocol, which is “40nm protocol”.

More specialty protocols for bases with different needs (than bases 1-8 in this example) can be added by clicking the “+” button. This will introduce another sub-amidite group that can be populated with more bases and a protocol. This can be done for every sinlge base if so desired.

IMPORTANT! The primary protocol and sub-amidite protocols must have matching section orders. The values within each section can be changed, but the order of injections cannot. For example, this is allowable:

Primary Protocol		
1 Rep	TCA	2
	Purge	5
2 Reps	Activator	1
	Amidite	
	Hold	60
	Purge	4

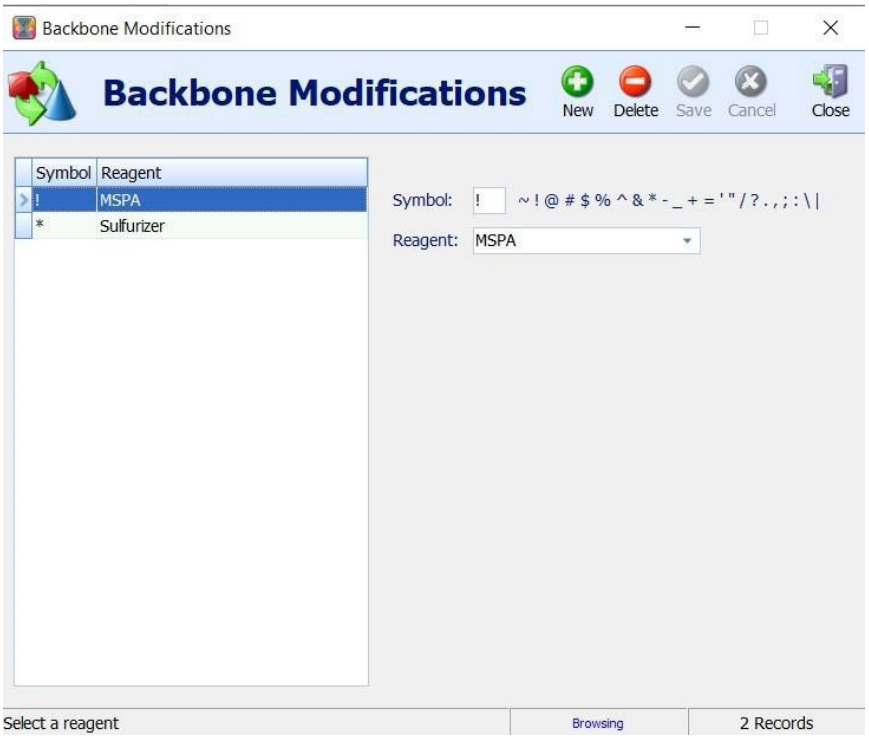
Sub-Amidite Protocol		
1 Reps	TCA	12
	Purge	10
4 Reps	Activator	3
	Amidite	
	Hold	180
	Purge	4

But this would *not* be allowable:

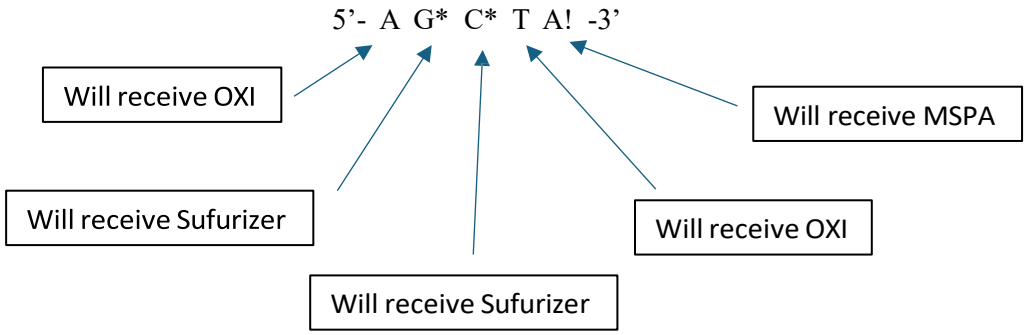
Primary Protocol		
1 Rep	TCA	2
	Purge	5
2 Reps	Activator	1
	Amidite	
	Hold	60
	Purge	4

Sub-Amidite Protocol		
4 Reps	Activator	1.3
	Amidite	
	Hold	180
	Purge	4
1 Reps	TCA	12
	Purge	10

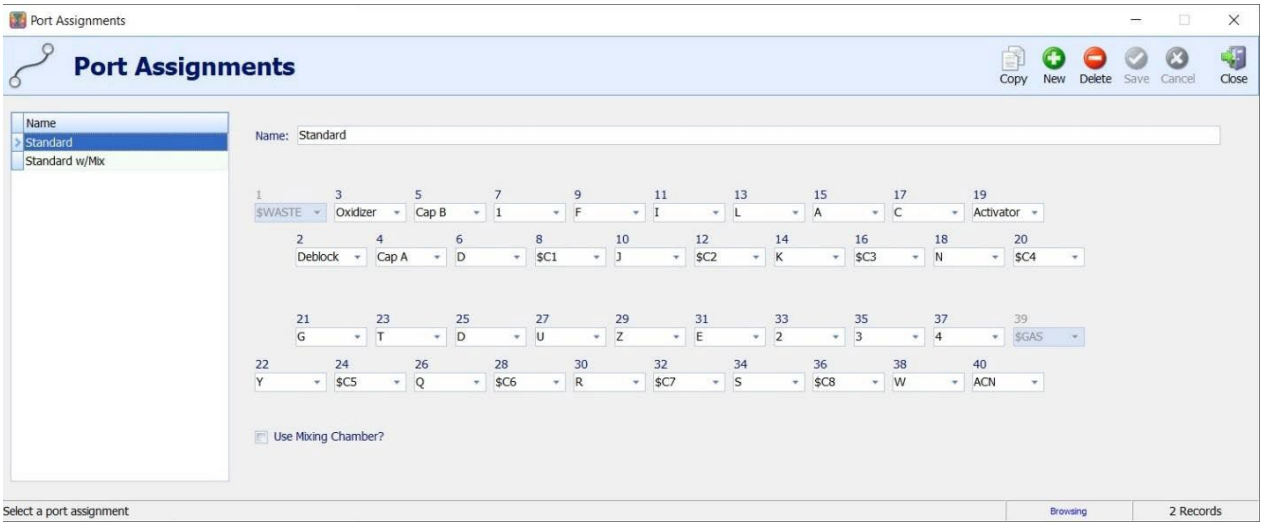
Backbone Modifications



- **Backbone Logic**
  - All reagents that will be used in the backbone of the oligo need to be listed in the Backbone Modification window and tied to a punctuation mark. The available options are listed on the screen.
  - Oxidizer is the default backbone. No punctuation mark is required to call this out.
  - To utilize these backbone modifiers, insert the punctuation mark associated with them into the sequence, place them where the would actually be in the oligo. For example:



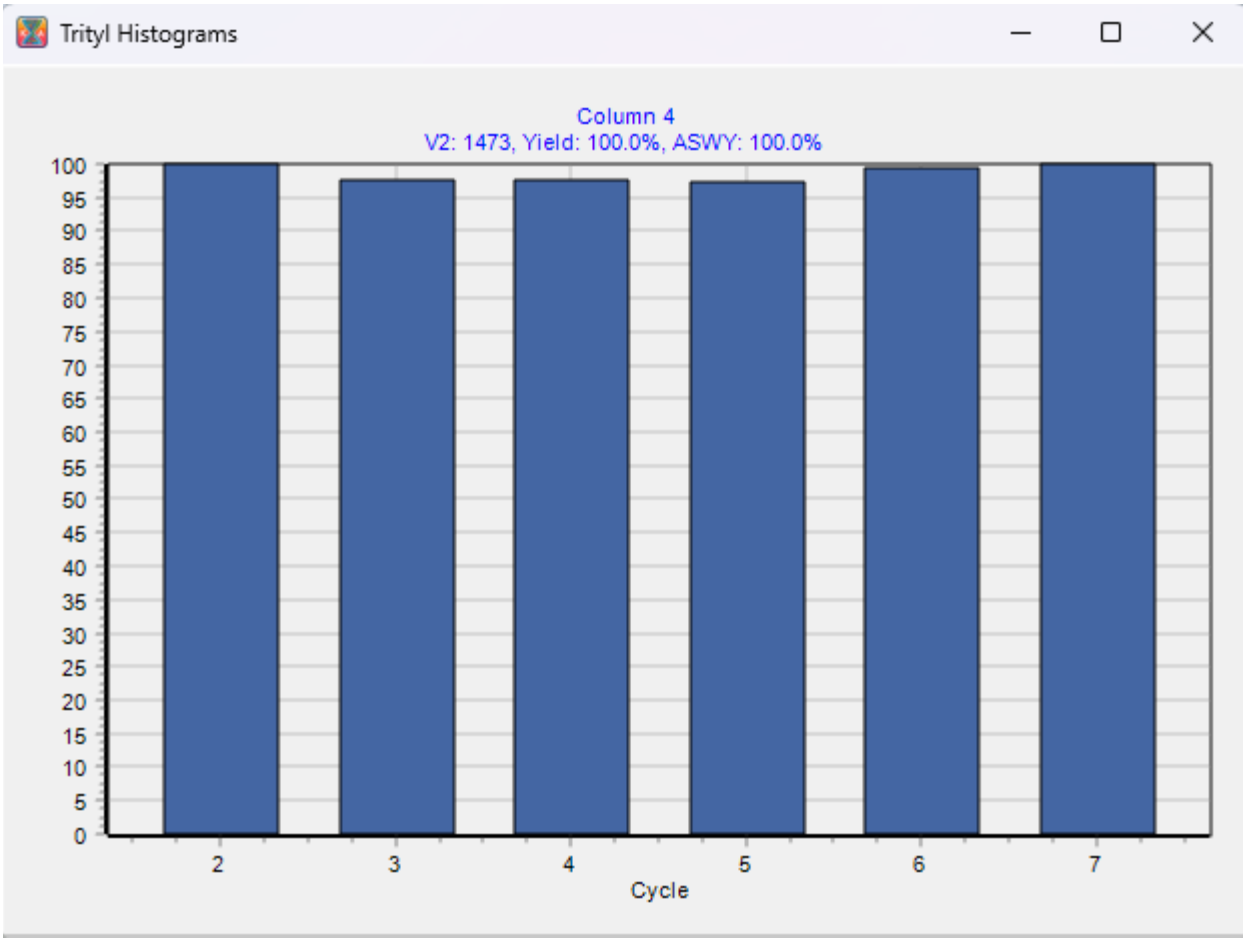
Port Assignments



The Port Assignments screen allows you to replumb the K&A.

- Each position contains a drop-down that allows the user to select any reagent or amidite to be in that position.
- Columns can also be replumbed to be additional reagent/amidite positions.
- Multiple assignments files can be created, but the one K&A currently uses must be assigned in the Configuration screen.
- The mixing chamber can be plumbed in for use anytime. If plumbed, select the “Use Mixing Chamber?” function. It will become an available selection.
- NOTE: Column positions cannot be reassigned to other locations.
- The same reagent/amidite cannot be listed in 2 locations at the same time.

Trityl Histograms



V2 Value

Our trityl monitor is a photometer that measures trityl levels in the cuvettes for each column individually. Measurements are taken at a wavelength of 470 nm (blue light), which provides the highest sensitivity for detecting trityl.

V2 represents the transmission measurement for the second trityl during synthesis. This value serves as the reference for all subsequent trityl measurements in the following cycles. Each subsequent transmission value is compared against V2.

The trityl monitor does not display an absolute, calibrated optical density (OD) or similar measurement; instead, it evaluates all measured trityl values relative to the V2 reference.

For the monitor chart, all values are converted into extinction values, ranging from approximately 1 (indicating very strong color with no transmission) to around 4095 (indicating full transmission with no color). A lower V2 value corresponds to a stronger color, which reflects a higher-quality coupling in the previous step.

It is crucial to ensure that the V2 reference is accurate. If the initial V2 reference is incorrect due to poor first coupling, the monitor may still produce a well-structured chart, but the resulting oligonucleotide will be of poor quality.

V2 also depends on factors such as the specific chemistry used, the dilution of trityl in the cuvette before measurement, and other synthesis conditions.

Typically, acceptable V2 values range between 1 and 100. However, you should compare the V2 range in your case with later oligo quality control (QC) results to determine the optimal V2 range for your synthesis conditions.

Total Yield

The total yield is determined by comparing transmission measurements across synthesis cycles. If the transmission value remains unchanged between cycles, the total yield is 100%. The trityl percentage is calculated using the following formula:

$$[1-(\text{LastTritylRead}-\text{TritylBaseline})/(4095-\text{TritylBaseline})]\times 100$$

In this formula:

- The **TritylBaseline** is the reference transmission value set after an early cycle.
- The **LastTritylRead** is the transmission measurement at the current cycle.
- **4095** represents the maximum possible sensor reading.

Since the formula includes a **"1 minus"** operation, the resulting percentage decreases as the **LastTritylRead** value increases toward 4095.

Example Calculations:

1. **Cycle 2 (TritylBaseline = 1700, LastTritylRead = 1700):**

$$[1-(1700-1700)/(4095-1700)]\times 100=100\%$$

Since there is no change in transmission, the total yield remains at **100%**.

2. **Cycle 20 (TritylBaseline = 1700, LastTritylRead = 3000):**

$$[1-(3000-1700)/(4095-1700)]\times 100=45.7\%$$

Here, the transmission has increased, indicating a decrease in trityl retention, and the total yield drops to **45.7%**.

As the **LastTritylRead** value approaches **4095**, the calculated yield percentage will approach **0%**, reflecting a near-complete loss of trityl signal.

ASWY

ASWY (Average Step-Wise Yield) represents the efficiency of each synthesis cycle. It is calculated by taking the current total yield and raising it to the power of **1 divided by the number of cycles**

Formula:

ASWY=[Total Yield^(1/Number of Cycles)] x 100
Where:

- **Total Yield** is the percentage of material remaining after a certain number of cycles (expressed as a decimal for calculation).
- **Number of Cycles** is the total number of synthesis steps completed.
- **ASWY (%)** represents the average coupling efficiency per cycle.

Example Calculations
Example 1: High Yield Process

- After **20 cycles**, the total yield is **45.7%** (**0.457 in decimal form**).
- The ASWY is calculated as:

ASWY=[(0.457)^(1/20)] × 100

ASWY≈97.45%
Interpretation:
Each cycle has a **97.45%** coupling efficiency on average.

Graph Zooming

Use the left mouse button to zoom in or out. Use the right-mouse button to scroll. Each chart is independent from the others when it comes to zoom and scroll.

Left click and drag down and to the right to select the part of the graph you want to zoom into. To zoom out, left-click and drag up and left.

Trityl Events

The way the protocol is written can influence the **fill and purge events** of the trityl monitor, which in turn affects the quality of the readings.

- The **first deblocking step** is always sent to the trityl monitor in its entirety.
- If there is **deblocking waste** that should be discarded instead of being sent to the monitor, those injections should be placed in a **subsequent section** of the protocol.
- In some cases, it may be beneficial to **add a deblocking step at the end of the cycle**. This ensures that the first deblocking injections are sent to waste rather than being measured by the monitor.

The trityl monitor is **always purged at the end of each cycle**.

- The duration of the purge is defined in the **Configuration screen**.

Instrument Operation

Overview

About This Chapter This chapter provides an overview of the K&A Oligo Synthesizer operation and maintenance procedures that you will perform most often.

Topic	See Page
Running a Synthesis	35
Shut Down Procedure	36
Air Compressor Maintenance	43
Changing Bottle Caps	45

Running a Synthesis

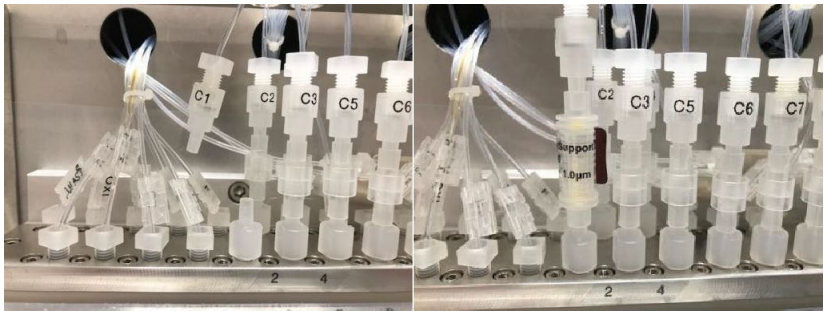
Preparing the Instrument

IMPORTANT! Ensure **high pressure** is applied to the instrument **before filling any reagent bottles**.

- Load fresh amidites and reagents onto the instrument.
- Connect all waste tubes to the designated waste container.
- Open the gas switches in the following order:
  - **High Pressure (HIGH):** 4.0 bar (60 psi)
  - **Low Pressure (LOW):** 0.4 bar (6 psi)
  - **Amidites and TET (A):** Open
  - **Reagents (R):** Open

Starting the Instrument

- Attach columns to the instrument.
  - NOTE: If some column positions are not in use, connect used columns to those positions to avoid reagent loss due to incorrect sequence-to-column assignments.



- In the "Run Sythesis" screen of the software, assign sequences to the appropriate columns.
- Select the desired protocol.
- In Manual Control, prime the required amidites and reagents.
- Click START to begin synthesis.

Completing a Synthesis

Once synthesis is completed on a position, the corresponding column may be removed.

The instrument will continue synthesis on remaining positions that are still active.

Refilling Bottles During a Run

When a reagent or amidite bottle is nearly empty:

1. Click PAUSE and wait for the signal and "Pause" status message.
2. Refill the bottle.
3. For amidite bottles, inspect the O-ring before closing.
4. If needed, press PRIME DNA and wait for priming to finish.
5. Click PAUSE again to resume synthesis.

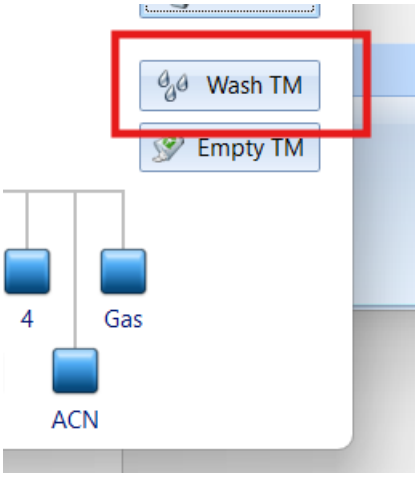
Maintenance

Shut Down Procedure

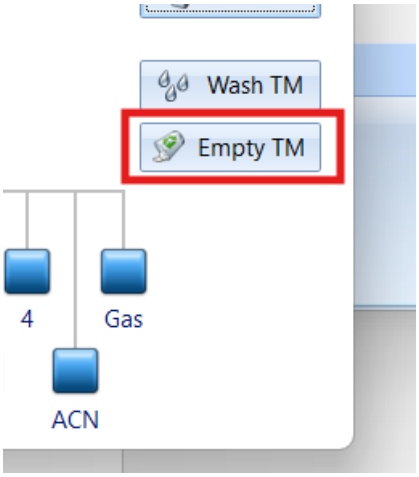
IMPORTANT! Always **shut down** and **open all bottles (amidites and reagents)** before closing the high-pressure system.

Perform the Shut Down Procedure when repairs are necessary.

1. Wash the trityl Monitors by selecting the “WASH TM” button in the Manual Control Screen

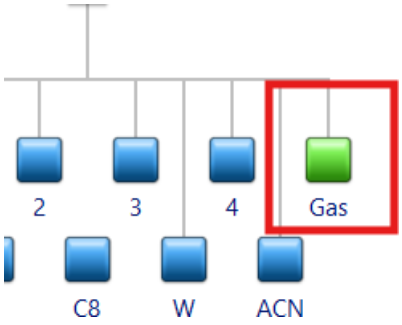


2. Empty the Trityl Monitors by clicking the “EMPTY TM” button in the Manual control screen of the software.

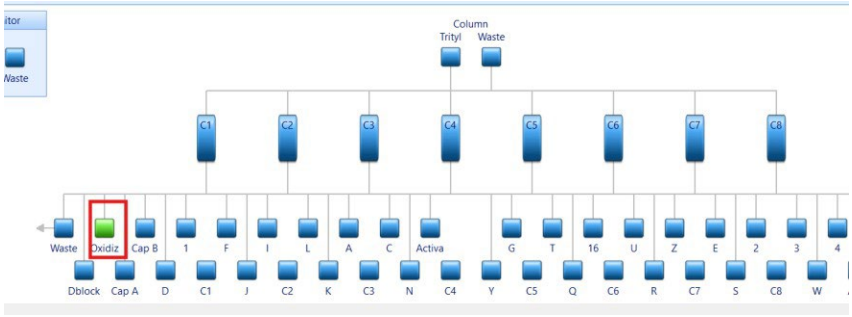


3. Close Amidite and Reagent Pressure Valves (Do not close the main Argon Pressure Valve!)

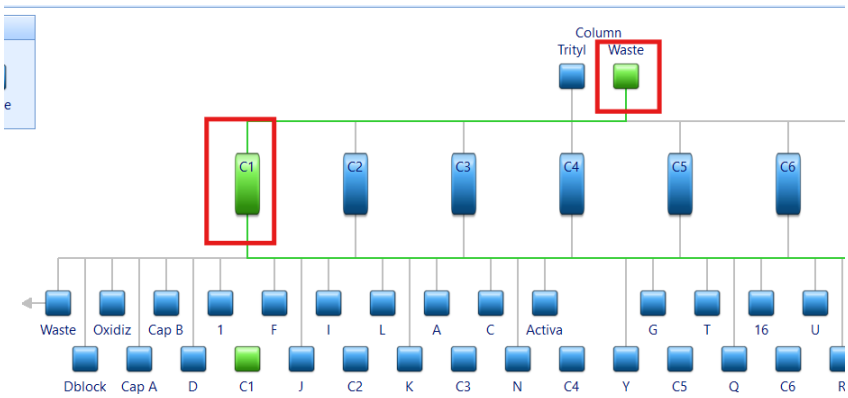
4. Loosen every bottle cap to depressurize all amidite and reagent bottles
5. If the instrument has a small vial adapter for 2ml bottles on the front, loosen the fittings that supply gas on either side
6. In the Manual Control Screen, Turn on the Gas Valve



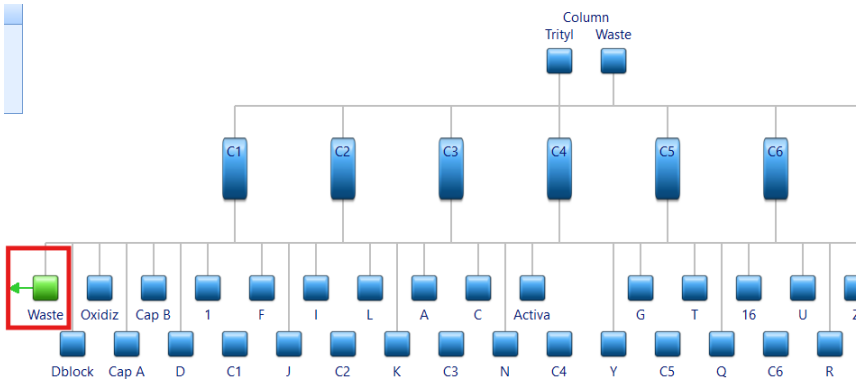
7. While the gas valve is on, one at a time fire each liquid valve for about 4-5 seconds or you are sure all liquid has entered the bottles



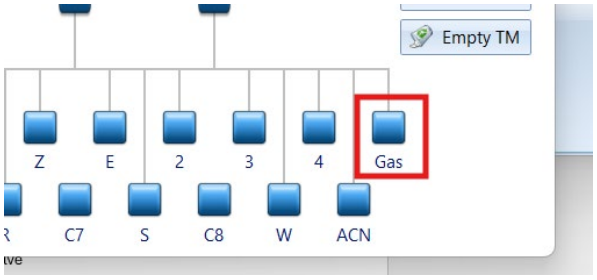
8. Fire each Column Valve+Waste valve one at a time to get rid of any excess liquid in the column waste lines



9. Fire the Main Waste Valve for 5-6 seconds or until you are sure all waste has left the lines



10. Turn off the Gas Valve



11. Place a cap on the amdities or Reagents if necessary
12. It is now safe to turn off the Main Argon valve and Compressor gas valve, if necessary.

Changing the Main Block Membrane (40-port)

Frequency: Every 4 months or as needed.

Steps:

1. Perform the Shut Down procedure.
2. Open all reagent/amidite bottles and close both high and low-pressure switches.
3. Remove the protective plate by unscrewing its two screws.
4. Disconnect reagent, amidite, and column luer-lock fittings.
5. Unscrew all M3 screws securing the valve block.
6. Clean all valve block components and install the new membrane.
7. Reassemble all parts. Refer to *Chapter 3.2: Submenu Control – Manual Control* for layout.
8. Close bottles, then open pressure switches in the following order:
   8.1. Low pressure (0.4 MPa)
   8.2. Amidites and reagents
9. Check all fittings for leaks.
10. Prime reagents and amidites.

Purge Air Compressor of Water (Weekly)

Purge the Regulator and Particle Filter (if applicable).

1. Close the Red Air Tap
2. In no particular order, press the conical relief valve at the base of the regulator and particle filter:

Purge the Compressor Water Reservoir:

1. Remove the cap from the waste tube (or the whole tube) from the tap for the water reservoir
2. Prepare a bottle to collect the waste from the reservoir
3. Open the tap for the water reservoir

NOTE: Open the tap slowly! Pressure may be significant!

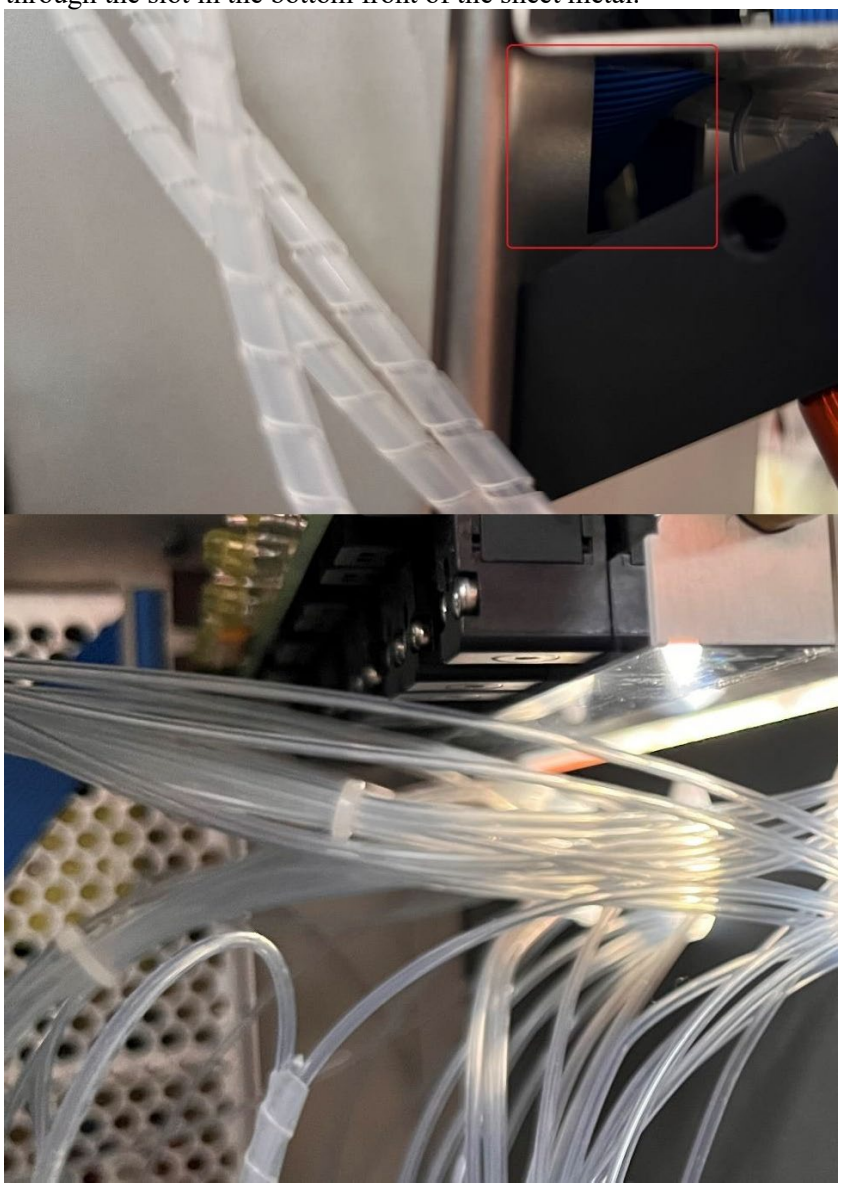
Clean Bottle Caps	Weekly
Clean Column Luer Lock Adapters	Monthly
Upper Valve Block Membrane Replacement (5x3)	Only replace if a blockage occurs (e.g., no flow through a column). Procedure is the same as for the main block.

Replacing Pneumatic Valves (As Needed)

1. Perform the **SHUT DOWN** procedure.
2. Open all bottles and close pressure valves.
3. Remove protective plate (main block only).
4. Remove the electronic board.
5. Replace the faulty valve (remove two M1.7 screws) and check the seal before reinstalling.
6. Reassemble the electronic board and all other components.
7. Reopen the pressure system (begin with 0.4 MPa).
8. Prime reagents and amidites.
9. **Note:** For valve replacement in the 5×3 block, access through the side doors.

Bottle Cap Change

1. Turn off the Argon Pressure to the instrument
2. Run the Liquid and gas lines for the bottle cap you wish to install through the slot in the bottom front of the sheet metal.



3. Attach the gas line (the line without the label) to any position on the gas manifold. This might require the removal of a plug. NOTE: Typically one gas manifold will be for the reagents and one or two gas manifolds will be for the amidites.

4. Pull the liquid line (the line with the label) through the hole closest to the position on the main manifold.

5. Attach the liquid fitting to the manifold and secure tightly to ensure no leaks
6. Re-pressurize the instrument
7. If liquid is in the bottle verify no leak is visible at the manifold or anywhere else.