K&A Labs Oligonucleotide Synthesizer

User Manual

Software: KA Labs Version 0.0.16.6

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Audience	Laboratory Operators & End Users

Revision History

Version	Date	Author	Description of Changes
v1.0	Apr 2026	H. Devoz	Split from combined manual; User Manual and Service Manual issued separately

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Table of Contents

How to Use This Manual

This User Manual covers everything you need to operate the K&A Labs Oligonucleotide Synthesizer on a day-to-day basis — from installation and software setup through running syntheses, monitoring results, and post-processing.

The K&A documentation set consists of two documents:

Document	Purpose
K&A Labs User Manual (this document)	Day-to-day operation: installation, software configuration, running syntheses, monitoring runs, post-processing, and basic troubleshooting.
K&A Labs Service Manual	Maintenance and servicing: compressor upkeep, membrane replacement, valve replacement, bottle cap changes, detailed troubleshooting, and service logs.

When this manual refers you to the Service Manual, it means the task at hand involves hardware maintenance or component replacement that is covered in detail there. Both manuals are intended for use by trained laboratory personnel — the Service Manual does not require a specialist service engineer.

> **NOTE**
> If you are setting up the instrument for the first time, start with Section 4 — Installation, then proceed to Section 5 — Software to configure the system before your first run.

1. Introduction

1.1 Overview

The K&A Oligonucleotide Synthesizer is an automated instrument designed for the synthesis of DNA, RNA, and modified oligonucleotides using pressure-driven reagent delivery.

The system performs stepwise nucleotide coupling on a solid support to produce customized oligonucleotide sequences. The instrument supports:

- Standard DNA synthesis
- RNA synthesis
- Backbone-modified oligonucleotides
- Custom synthesis protocols and modified nucleotides

The K&A system uses a fully pressure-driven fluidic architecture, eliminating the need for internal liquid pumps. Argon pressure drives reagent delivery while pneumatic valves control routing through the manifold to the synthesis columns.

Integrated software provides full control of synthesis operations including:

- Sequence management
- Protocol creation and editing
- Reagent configuration
- Real-time run monitoring
- Data logging and analysis

1.2 Intended Audience

This manual is intended for laboratory personnel operating the synthesizer on a day-to-day basis. Users are expected to have familiarity with:

- Basic Windows operating system usage
- Standard laboratory practices and safety procedures
- Oligonucleotide synthesis workflows

> **NOTE**
> For maintenance procedures including membrane replacement, valve replacement, and compressor servicing, refer to the K&A Labs Service Manual.

1.3 Instrument Identification

Record the instrument information below for future reference. This information may be required when contacting Sierra BioSystems technical support.

Instrument Model	
Serial Number	
Software Version	
Installation Date	

2. Safety

2.1 User Attention Words

Term	Meaning
NOTE	Useful information that improves understanding or performance
IMPORTANT	Information required for proper instrument operation
CAUTION	Risk of minor or moderate injury if precaution is not taken
WARNING	Risk of serious injury or death if warning is not heeded
DANGER	Immediate risk of serious injury or death

2.2 Chemical Hazard

⚠ WARNING

Some chemicals used with the K&A instrument are potentially flammable. Read and understand all Material Safety Data Sheets (MSDSs) before storing, handling, or working with any chemicals. Minimize contact with and inhalation of chemicals. Wear appropriate PPE at all times: safety glasses, gloves, and a lab coat. Do not leave chemical containers open. Use only in a well-ventilated area or fume hood. Comply with all applicable local, state, and national regulations for chemical storage, handling, and disposal.

2.3 System Pressurization

⚠ WARNING

Always depressurize the system fully before disconnecting any tubing, fittings, or components. Do not operate the instrument outside its intended use as described in this manual. Ensure all personnel are trained before operating the instrument.

2.4 Before Operating the Instrument

Ensure that everyone involved with instrument operation has:

- Received instruction in general laboratory safety practices
- Received instrument-specific safety training
- Read and understood all relevant MSDSs

> **⚠ CAUTION**
> Avoid using this instrument in any manner not specified by Sierra BioSystems. The instrument has been designed to protect the user, but this protection may be compromised if the instrument is used improperly.

2.5 Laboratory Requirements

Parameter	Requirement
Temperature	16–22°C (operational range 10–40°C)
Humidity	Up to 99%
Altitude	< 2000 m
Power	120–240 VAC, 50/60 Hz
Gas — High Pressure	Compressed air, oil- and water-free; 65–80 psi (0.45–0.55 MPa)
Gas — Low Pressure	Argon (99.99%); 30–40 psi (0.21–0.28 MPa)
Environment	Non-conductive pollutants only

3. System

3.1 System Description

The K&A Synthesizer performs stepwise nucleotide coupling to produce oligonucleotides linked to a solid support. It is engineered for maximum flexibility, supporting standard DNA/RNA synthesis as well as heavily modified oligonucleotides and custom synthesis protocols.

Key features include:

- Fully pressure-driven fluid delivery — no internal pumps or motors
- Flexible reagent and amidite configuration with interchangeable position assignments
- Support for custom, modified, and backbone-modified oligonucleotides
- Advanced software with real-time monitoring, protocol customization, and data logging

Figure 3-1: K&A Labs Oligonucleotide Synthesizer

3.2 System Architecture

The instrument is organized into three integrated subsystems:

Fluidics

Manages reagent delivery from pressurized bottles through the valve manifold to the synthesis columns and waste system.

Pneumatics

Argon gas at low pressure drives fluid movement through the system. Compressed air at higher pressure actuates the pneumatic valves that control the sealing membrane.

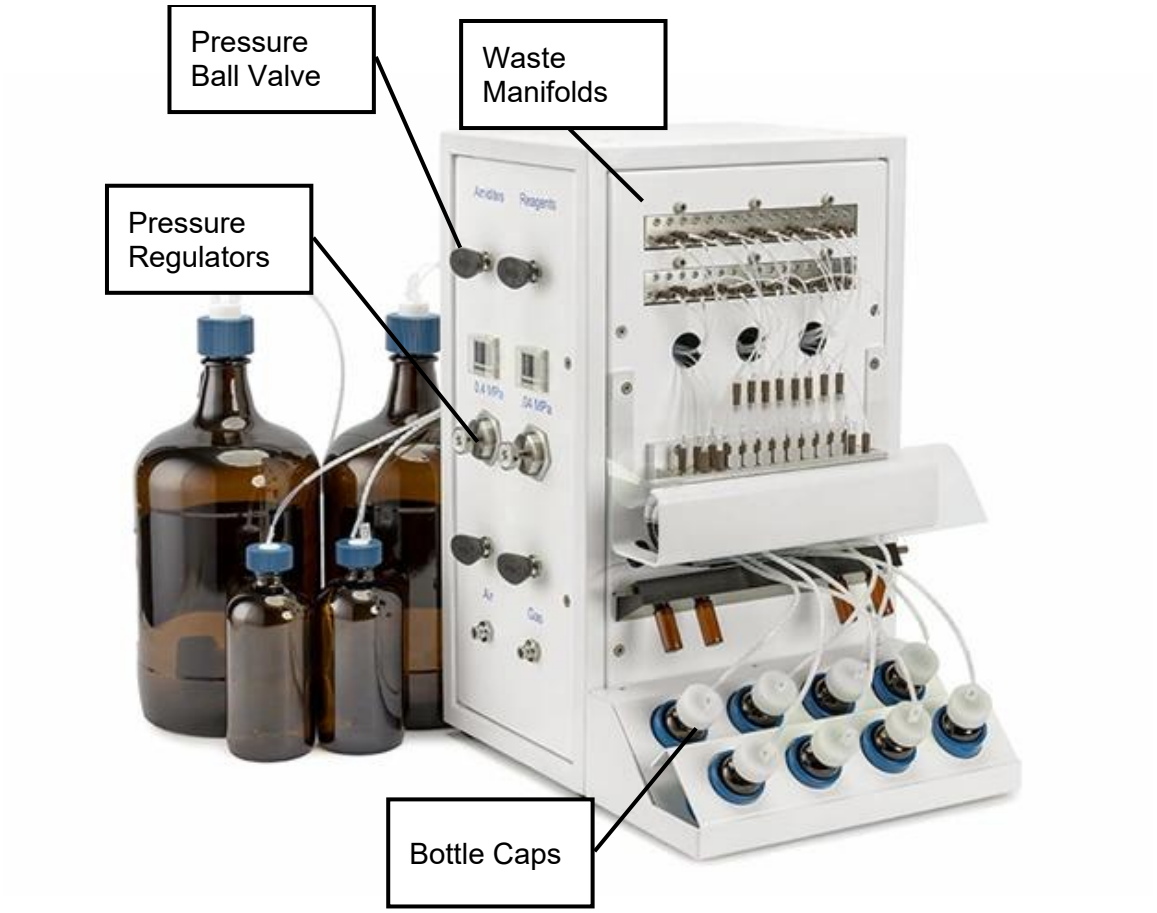
Electronics

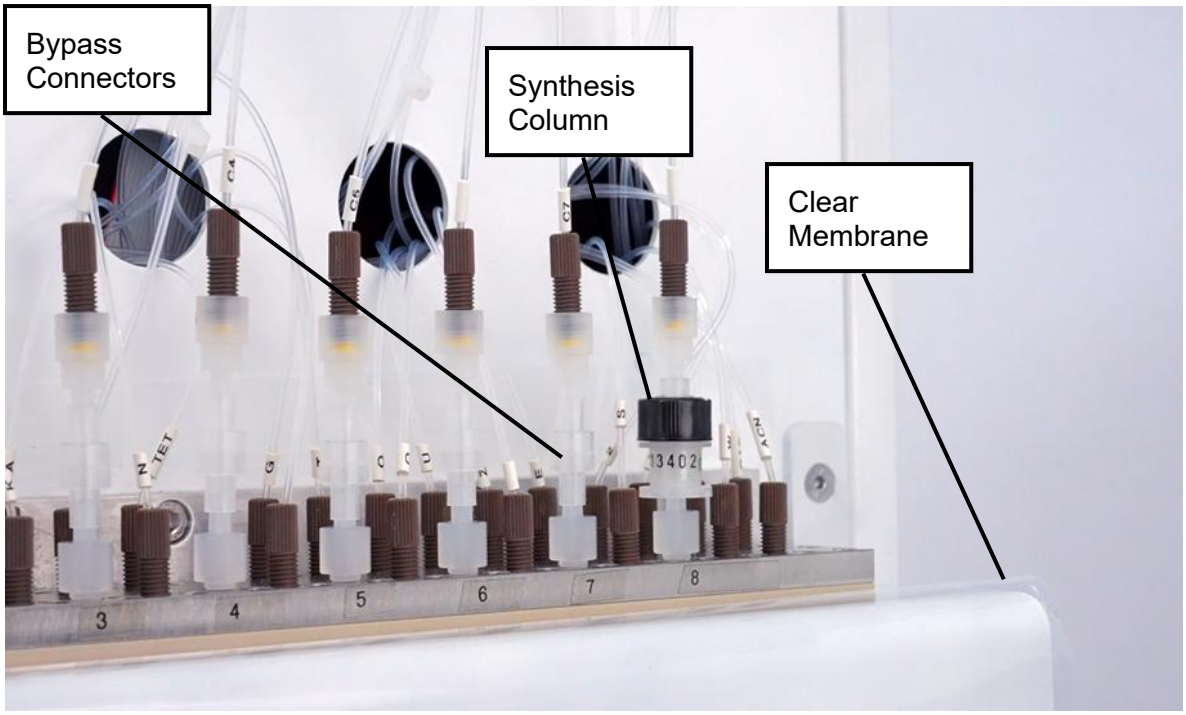
Controls valve actuation, communicates with the KA Labs software, and interfaces with the trityl monitor sensor.

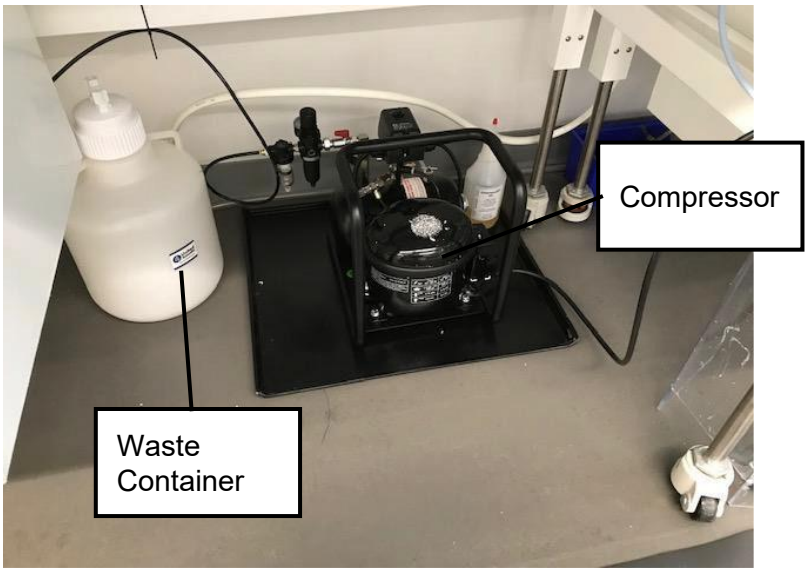
> **NOTE**
> The system uses argon pressure and gas-actuated valves to control fluid flow. There are no liquid pumps or electrically actuated solenoid valves in contact with reagents, minimizing chemical exposure to mechanical components.

3.3 Major Components

Component	Function
Pneumatic Valves	Control fluid routing through the manifold
Pressure Regulators	Maintain correct gas pressure for fluid delivery and pneumatic control circuits
Pressure Ball Valves	Manual valves used to open and close the argon supply to reagent and amidite bottle groups
Bottle Caps	Sealed caps fitted with gas and liquid line connections that pressurize reagent and amidite bottles
Sealing Membrane	Flexible membrane that blocks fluid flow when pressurized and opens during valve actuation
Main Block	Central manifold block that routes reagent and amidite delivery to the synthesis columns
Synthesis Columns	Reaction vessels where oligonucleotide coupling occurs
Trityl Monitor	Optical sensor that measures coupling efficiency by detecting trityl absorbance at 470 nm
Waste Manifolds	Tubing network that routes waste fluids away from the columns
Waste Container	Collects waste fluids generated during synthesis
Air Compressor	Supplies compressed air used to actuate pneumatic valves


Pressure
Ball Valve
Waste
Manifolds
Pressure
Regulators
Bottle Caps

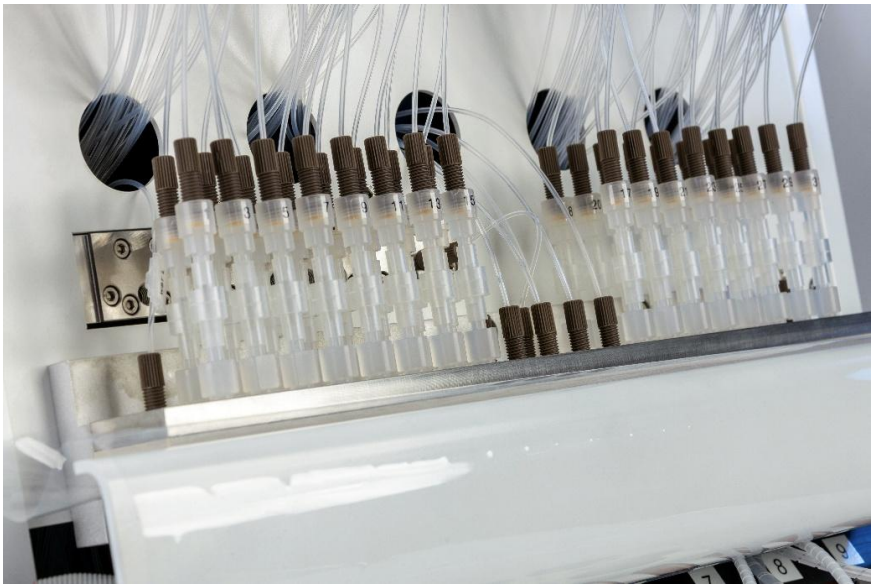

Bypass
Connectors
Synthesis
Column
Clear
Membrane

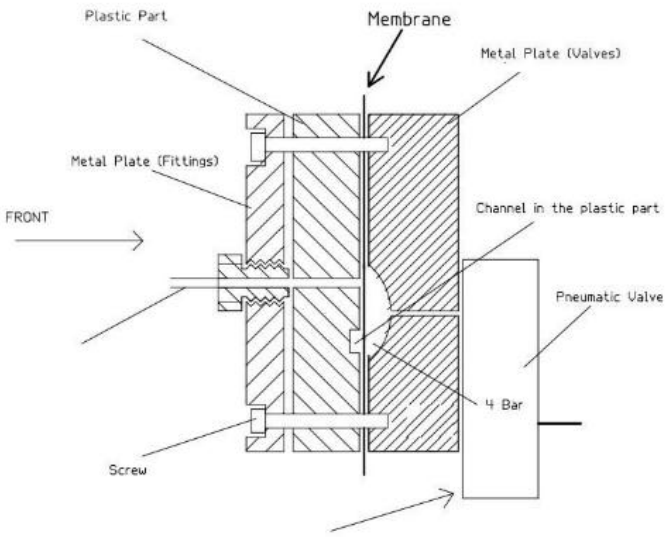


3.4 Membrane Operation

The K&A synthesizer uses a flexible membrane valve system to control fluid delivery through the main valve block.

Reagents stored in pressurized bottles flow toward the membrane but cannot enter the manifold while the membrane is held closed by compressed air. When a pneumatic valve is activated, the compressed air pressure on that channel is temporarily released, allowing the pressurized reagent to push the membrane open and flow into the common manifold toward the synthesis column. When the valve closes, compressed air pressure reseals the membrane and stops fluid flow.





This design provides:

- Precise reagent delivery
- Reliable switching between reagents
- Minimal cross-contamination between steps

3.5 Key Operating Specifications

Parameter	Value
Maximum Power	~240 W
Recommended Low-Pressure Setting	0.04 – 0.07 MPa (argon)
Recommended High-Pressure Setting	0.30 – 0.40 MPa (compressed air)
Fluid Control	Pneumatic membrane valve system
Trityl Detection Wavelength	470 nm
Fuse	3.15 A (slow)

4. Installation

4.1 Pre-Installation Requirements

4.1.1 Site Requirements

- Stable laboratory bench with a minimum carrying capacity of 200 lbs
- Adequate workspace around the instrument for safe operation and maintenance access
- Access to power, gas connections, and Ethernet
- Proper ventilation; use within a fume hood is strongly recommended

4.1.2 Electrical Requirements

Location	Voltage (VAC)	Frequency	Amperage
USA / Canada	120 ± 10%	50/60 Hz	2 A
Europe	240 ± 10%	50/60 Hz	1 A
Australia	240 ± 10%	50/60 Hz	1 A
Japan	110 ± 10%	50/60 Hz	2 A

A standard grounded outlet on a dedicated 15A circuit is required for USA, Canada, and Japan installations. A 10A circuit is required for Europe and Australia installations.

IMPORTANT
Poor grounding or unstable power can cause communication failures and unreliable instrument operation. A dedicated grounded line is strongly recommended.

4.1.3 Gas Requirements

Argon (Low Pressure)

- Purity: 99.99%
- Pressure: 30–40 psi (0.21–0.28 MPa)

Used for fluid delivery from reagent and amidite bottles.

Compressed Air (High Pressure)

- Pressure: 65– 80 psi (0.45 –0.55MPa)
- Air must be clean, dry, and oil-free

Compressed air is used for pneumatic valve actuation. An air compressor is supplied with the instrument. A filtered laboratory compressed air source may also be used provided it delivers

clean dry air, remains within the specified pressure range, and does not experience pressure shutdowns or fluctuations.

IMPORTANT
High pressure must be applied to the instrument before filling or pressurizing any reagent or amidite bottles.

⚠ WARNING
Never turn off the high-pressure air supply while reagent or amidite bottles remain pressurized. Always depressurize bottles first by loosening their caps before closing the high-pressure supply. Closing high pressure while bottles remain pressurized may cause uncontrolled fluid movement, reagent spills, and potential instrument damage.

4.2 Unpacking & Inspection

Carefully unpack all components and verify the following items are present:

- K&A Synthesizer
- Computer with Windows OS and KA Labs software pre-installed
- Monitor, keyboard, and mouse
- Power cords (4)
- Ethernet cable
- Air compressor
- Waste Carboy (if requested)

Inspect all components for shipping damage, loose fittings, or missing parts. Document any damage with photographs before proceeding.

⚠ WARNING
Do not install or power the system if visible damage is present. Contact Sierra BioSystems support before proceeding.

4.3 Installation Steps

1. Place the instrument securely on the laboratory bench, centered in both width and depth.
2. Confirm all four corners of the instrument sit flat on the bench surface.
3. Place the computer beside the synthesizer and connect the monitor, keyboard, and mouse.

Figure 4-1: Instrument placement and computer setup

4. Connect the Ethernet cable between the K&A Ethernet port and the computer Ethernet port.
5. Connect the instrument power cord to the rear panel of the instrument, then plug it into a grounded outlet.
6. Place the waste tubing ends into the waste container.

7. Turn on the instrument using the main power switch on the side panel.
8. Turn on the computer.
9. Prepare the Compressor for Installation. See the K&A Labs Service Manual — Appendix A for details.
10. Connect gas lines: Argon to the Low-pressure inlet, Compressed air to the High-pressure inlet.
11. Turn on the high-pressure source (air compressor).

Figure 4-3: Gas line connections

12. Confirm Compressed Air (high pressure) regulator is within the following range: 0.30–0.40 MPa.
13. Connect reagent bottles. Bottle caps for reagents will be connected to the labeled Manifold on the back. Liquid lines will be on the right, gas lines on the left.
14. Place amidite or empty bottles to their respective bottle caps.
15. Turn on the argon low-pressure supply.
16. Confirm Argon (low pressure) is within the following range: 0.04–0.07 MPa.
17. Verify that no leaks are present at fittings or tubing connections. See Section 8.2 for pressure leak identification.
18. Launch the KA Labs software from the desktop icon.

5. Software

5.1 Software Overview

KA Labs software is the primary interface used to configure the instrument, design synthesis protocols, and run oligonucleotide syntheses. The software is installed on the dedicated instrument computer and communicates with the synthesizer via Ethernet.

Core functions include:

- Running and monitoring synthesis
- Designing and customizing synthesis protocols (Standard and Meta)
- Managing sequences (import, export, and manual entry)
- Priming reagent and amidite lines
- Estimating reagent volume consumption before a run
- Calibrating and checking flow rates
- Manual valve control for diagnostics and maintenance
- Data logging and backup/restore

5.2 Interface Overview

The main toolbar is divided into two functional areas:

- Synthesis — Operational functions used during routine synthesis work: Run Synthesis, Protocol Writer, Manual Control, Priming, Protocols, Sequences
- Setup/Utilities — Configuration and system setup tools: Configuration, Reagents/Amidites, Port Assignments, Backbone Modifications, Flow Rates, Manage Servers, Backup/Restore

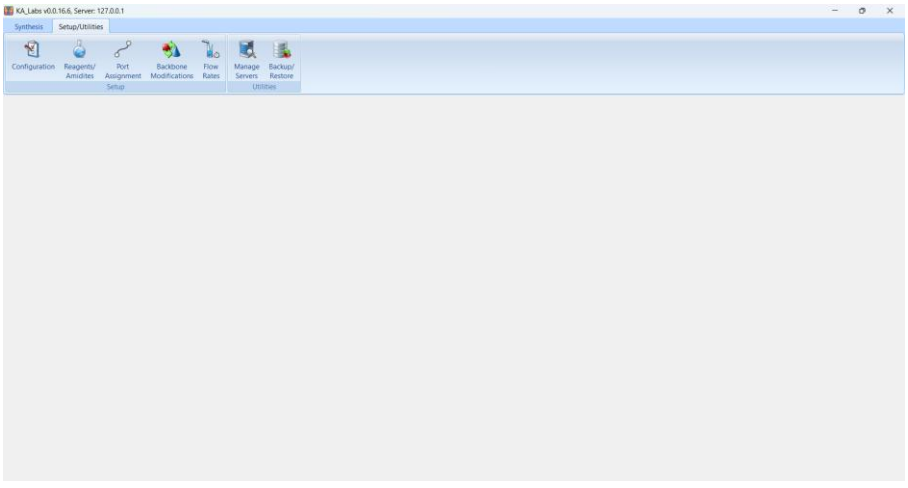


Figure 5-1: KA Labs Software — Main Toolbar

5.3 Instrument Configuration

5.3.1 Configuration

> **NOTE**
> Most users will not need to modify these settings during routine operation. The Configuration screen is typically set up once during installation.

Key settings include:

- **Port Assignment** — Selects the active Port Assignment used for the instrument.
- When Not Connected, Act As — Allows selection of an instrument model to simulate when no hardware is connected. Useful for building protocols offline.
- **Empty Trityl Monitor Time** — Sets the duration (in seconds) that argon gas is sent to the trityl monitor vials during the Empty TM function.

> **IMPORTANT**
> The recommended Empty TM time is 20 seconds for most instruments. For H8-VLS instruments, set to 40 seconds. Using too short a time may leave residual liquid in the vials and affect trityl readings.

Miscellaneous options:

- **Dual Column Injection** — When enabled, two columns can receive amidite simultaneously. Not recommended under normal operating conditions.
- **Mid-Synthesis Priming** — When enabled, automatically primes each valve the first time it is used within a synthesis section. Must be enabled here before it will take effect. See Section 5.5.1 — Priming for details.
- **Reverse Purge Columns (H8-VLS only)** — Defines how many columns will receive a reverse purge simultaneously.

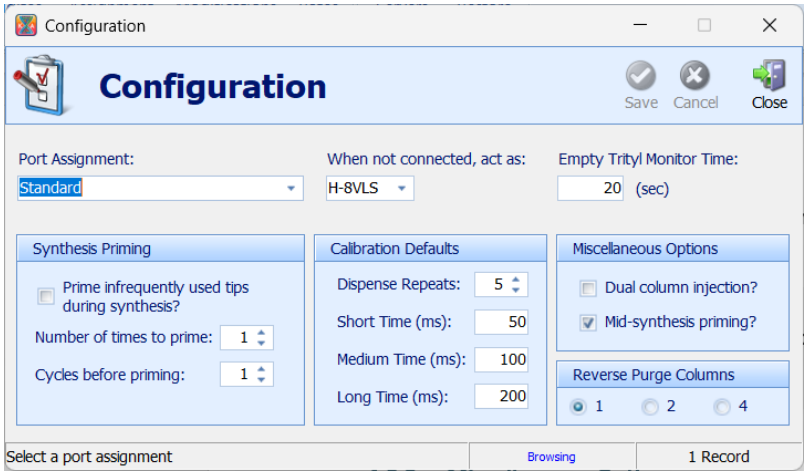


Figure 5-2: Configuration Screen

5.3.2 Reagents/Amidites

> **IMPORTANT**
> Each reagent must be defined here before it can be assigned to a port or used in a protocol.

The Reagents/Amidites screen is used to define every fluid plumbed to the instrument. This screen is typically set up during initial configuration and only needs to be updated when adding new reagents or amidites.

Field	Description
Name	Full name of the reagent or amidite (e.g., 'Oxidizer', 'A'). Displayed in logs and exported files.
Short Name	Abbreviated identifier used in the software interface (e.g., 'OXI', 'A').
Mix List	Used for wobble or degenerate bases only. Leave blank for all standard reagents and amidites.
Amidite?	Checkbox identifying the entry as an amidite. Backbone modifiers must NOT be checked as amidites.
Not Dispensed Color	Color displayed in Run Synthesis Color Display when queued but not yet dispensed.
Dispensed Color	Color displayed after the fluid has been dispensed in the current cycle.

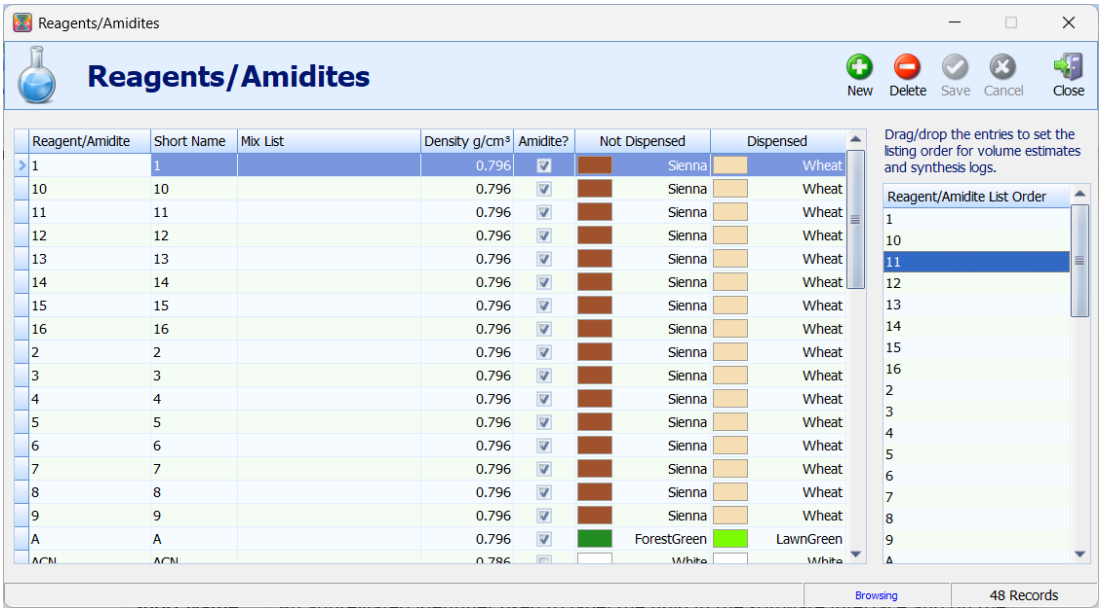


Figure 5-3: Reagents/Amidites Screen

IMPORTANT

Any reagent intended for use as a backbone modification must not have the 'Is Amidite' checkbox selected. If marked as an amidite, it will not be available in the Backbone Modifications screen.

5.3.3 Port Assignments

The Port Assignments screen defines which reagent or amidite is connected to each physical port on the instrument. This screen should always reflect the physical bottle configuration.

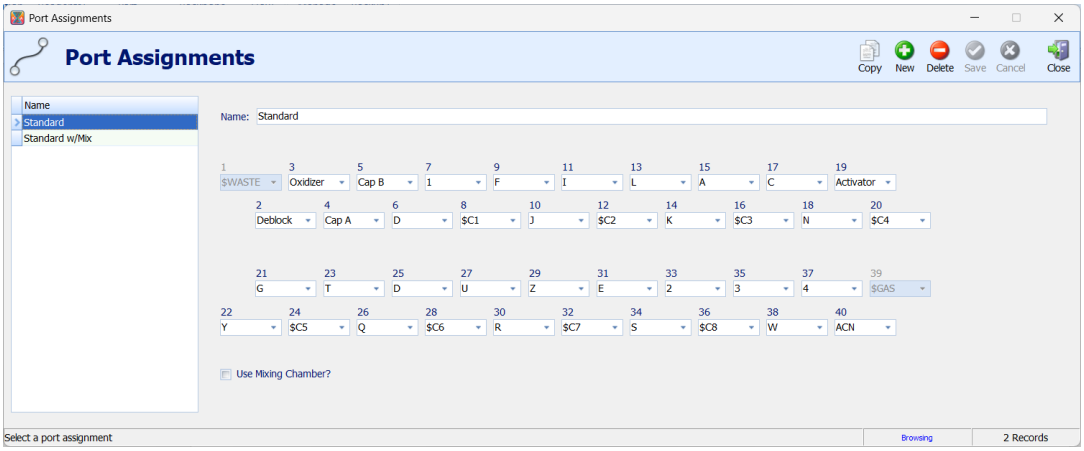


Figure 5-4: Port Assignments Screen

IMPORTANT

The active Port Assignment file must be selected in the Configuration screen. Saving a new file does not automatically make it active. Port Assignments must be updated any time a bottle is moved or a line is replumbed.

NOTE

The same reagent or amidite cannot be assigned to more than one port at the same time.

5.3.4 Backbone Modifications

The Backbone Modifications screen defines non-standard backbone linkages that can be called out within a sequence string. Each backbone modification pairs a reagent with a punctuation symbol. When that symbol appears in a sequence, the software substitutes the assigned backbone reagent in place of the default oxidizer.

To create a backbone modification: select the reagent from the dropdown, assign a punctuation symbol (one character from: ~!@#$%^&*-_+='"/?.,;:|\), then save the entry.

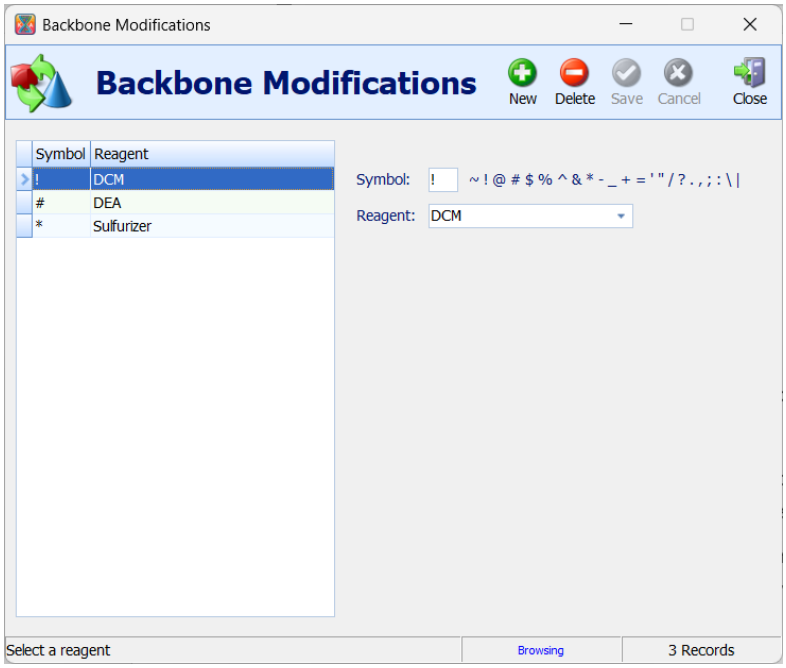


Figure 5-5: Backbone Modifications Screen

> **NOTE**
> Oxidizer is always the default backbone and does not require any symbol in the sequence string.

5.4 Protocol and Sequence Setup

5.4.1 Protocol Writer

The Protocol Writer is accessed from the Synthesis tab and is used to create and edit both Standard Protocols and Meta Protocols. Changes made here are saved to the database and are immediately available for use in synthesis runs.

> **NOTE**
> Zero values in the Time column cause that step to be skipped entirely. This is useful for bypassing purge steps when liquid must remain in the column.

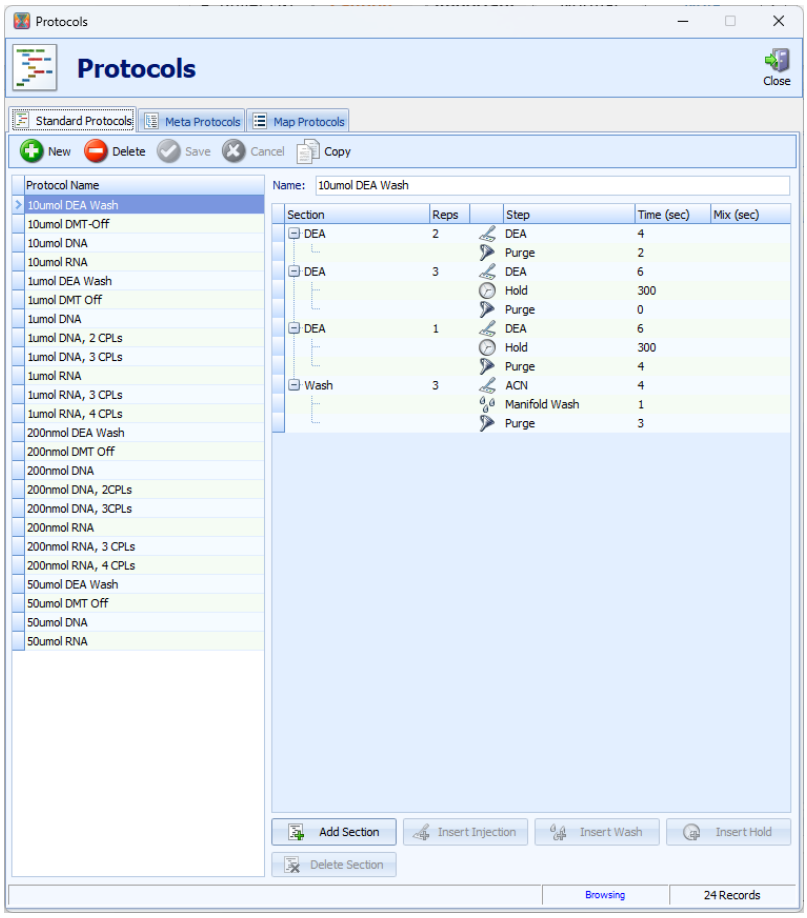


Figure 5-6: Protocol Writer

5.4.2 Standard Protocols

Standard protocols define the step-by-step synthesis cycle applied to columns and bases. Each protocol is organized into Sections containing dispense steps and supporting actions.

Element	Description
Sections	Contains all actions for a chemistry step. Typically includes a dispense, a purge, and any holds or manifold washes.
Reps	Number of repetitions for the section. All steps within the section will be repeated.
Dispense	Delivers a reagent or amidite for a defined time (seconds). Maximum 2 dispenses per section, in sequential order.
Mix	When two dispenses are listed sequentially, alternates between the two reagents for a set time.
Hold	A timed waiting period with no action, allowing the reaction to proceed on the support.
Wash (Manifold Wash)	Sends ACN through the manifold followed by gas. Recommended for viscous liquids such as Oxidizer and Sulfurizer.

Purge	Timed gas command to push liquid out of the column from bottom to top. Recommended for scales under 10 umol.
Reverse Purge (VLS only)	Pushes liquid from top through bottom. Recommended for larger scales over 10 umol in syringe-style columns.

> **NOTE**
> Zero values in the Time column cause that step to be skipped entirely. This is useful for bypassing purge steps when liquid must remain in the column, or for skipping injections for specific bases within a bank.

Available editing functions in the Protocol Writer:

Function	Description
Add Section	Adds a section to the current protocol. Multiple sections can be created for the same dispense if desired.
Add Injection	Inserts a dispense step into the section. Maximum 2 injections per section. A dropdown allows selection of the reagent (must not be marked as an Amidite).
Add Wash	Inserts a manifold wash into the current section. Multiple washes can be added.
Add Hold	Inserts a hold into the currently selected section. Multiple holds can be added.
Delete Section	Deletes the currently selected section from the protocol.
Purge / Rev Purge (VLS only)	Selecting the Purge step allows toggling between Purge and Reverse Purge.

5.4.3 Meta Protocols

Meta Protocols link Standard Protocols together to automate case-dependent operations across a full synthesis run. They define:

- Pre-Process Protocol — Runs before synthesis begins (e.g., pre-synthesis wash, extra deblock).
- DMT-Off Protocol — Runs immediately after synthesis completes for final DMT removal.
- Post-Process Protocol — Runs after DMT-Off (e.g., DEA treatment, additional DCM rinse).
- Base-Specific Protocols — Defines which protocol applies to each amidite.
- Special Protocols — Defined on a per-amidite basis within Base Protocols.
- Cycle-Specific Protocols — Determines which protocol is used depending on the base number.

IMPORTANT
The synthesizer will not automatically remove the final DMT group unless the DMT-Off protocol is specified both in the Meta Protocol and in the sequence file settings.

5.4.4 Sequences

Sequences are entered and managed in the Sequences window. All sequences must be written, imported, or copied into this window before they can be used in a synthesis run.

Field	Description
Name	A unique identifier for the sequence, displayed in the software, run log, and exported files.
DMT Off	Enables the DMT-Off protocol within the Meta Protocol for this sequence.
Univ	Indicates the use of a universal support. When deselected, the 3' base is skipped.
Pre / Post	Enables Pre-Process and Post-Process protocols for this sequence.
Sequence 5'→3'	The sequence as it will be synthesized.

Formatting rules: Single-character base names are listed sequentially. Multi-character names must be bracketed (e.g., [FAM]). Backbone punctuation marks must be included. Input is not case-sensitive.

Sequences can be imported and exported as .xlsx or .csv files. Sierra BioSystems recommends exporting a test sequence first to use as a formatting template before importing a batch.

The import file must contain the following columns in this order:

Column	Field	Example	Notes
A	Name	MyOligo_001	Unique identifier. No special characters.
B	Sequence (5'→3')	AGCT*AGCT[FAM]	Single-char bases sequential. Multi-char in brackets. Backbone symbols between bases.
C	DMT Off	TRUE	TRUE or FALSE. Removes final DMT group if TRUE.
D	Universal Support	FALSE	TRUE or FALSE. If FALSE, the 3' base is skipped.
E	Pre-Process	FALSE	TRUE or FALSE. Enables pre-process protocol.
F	Post-Process	FALSE	TRUE or FALSE. Enables post-process protocol.

> **NOTE**
> Input is not case-sensitive — [ma], [mA], [MA], and [Ma] are all treated identically. Export a sequence from the software first to use as a template before importing a large batch.

5.5 Preparing the Instrument

5.5.1 Priming

> **NOTE**
> Priming is recommended before every run, after replacing any reagent or amidite bottle, and after any extended idle period. It helps eliminate dead volume in the lines and ensures the first column receives the correct reagent volume.

The Priming screen is accessed from the Synthesis tab. Enter a dispense time in milliseconds (350 ms is recommended as a starting duration for most lines), select the valve, and select the prime mode.

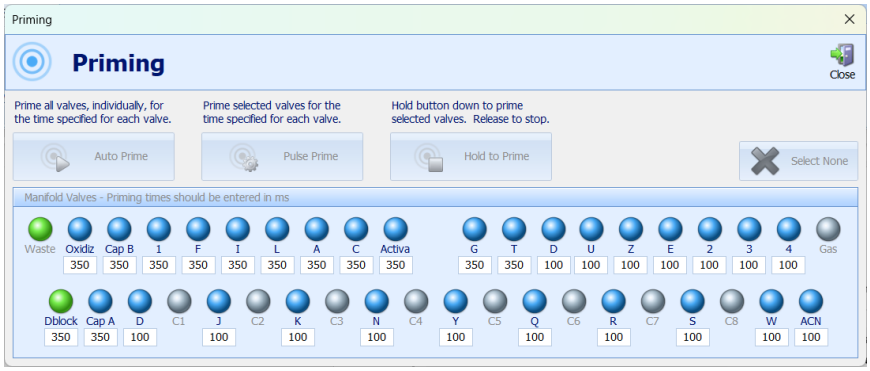


Figure 5-7: Priming Screen

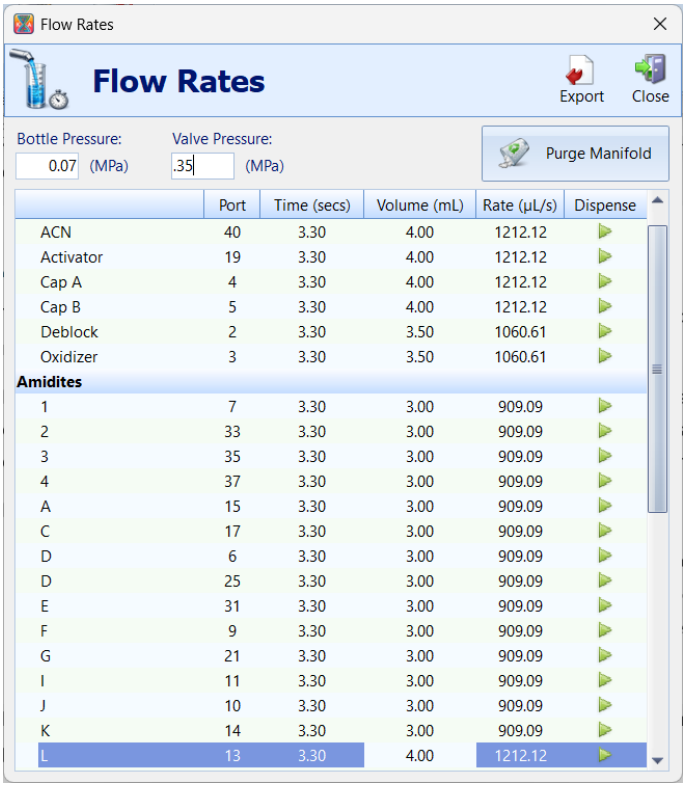
Mode	Description
Auto Prime	Fires each valve that has a time value entered, sequentially, for its set duration. Useful for priming multiple lines before a run.
Pulse Prime	Fires only the selected valve(s) once for the entered duration.
Hold to Prime	Opens the selected valve(s) and holds them open for as long as the button is pressed. Useful for visual confirmation of flow.

Mid-Synthesis Priming automatically primes a valve the first time it is used within a synthesis section. It must be enabled in the Configuration screen before it will take effect.

5.5.2 Flow Rates

The Flow Rates screen (Setup/Utilities tab) is used to calibrate and record the measured flow rate for each reagent and amidite line. These values are used by the software to calculate estimated reagent consumption.

Flow rates should be checked approximately once per month, when a discrepancy is suspected, after any valve replacement or tubing change, and when Estimate Volume results appear inaccurate.



	Port	Time (secs)	Volume (mL)	Rate (µL/s)	Dispense
ACN	40	3.30	4.00	1212.12	
Activator	19	3.30	4.00	1212.12	
Cap A	4	3.30	4.00	1212.12	
Cap B	5	3.30	4.00	1212.12	
Deblock	2	3.30	3.50	1060.61	
Oxidizer	3	3.30	3.50	1060.61	
Amidites					
1	7	3.30	3.00	909.09	
2	33	3.30	3.00	909.09	
3	35	3.30	3.00	909.09	
4	37	3.30	3.00	909.09	
A	15	3.30	3.00	909.09	
C	17	3.30	3.00	909.09	
D	6	3.30	3.00	909.09	
D	25	3.30	3.00	909.09	
E	31	3.30	3.00	909.09	
F	9	3.30	3.00	909.09	
G	21	3.30	3.00	909.09	
I	11	3.30	3.00	909.09	
J	10	3.30	3.00	909.09	
K	14	3.30	3.00	909.09	
L	13	3.30	4.00	1212.12	

Figure 5-8: Flow Rates Screen

IMPORTANT
Flow rate values must be entered and current for the Estimate Volume feature to produce accurate results.

5.6 Running a Synthesis

Typical synthesis workflow:

1. Load sequences
2. Select protocol
3. Confirm column configuration
4. Run Estimate Volume
5. Click Start

5.6.1 Run Synthesis Screen

The primary operating screen. Users pair sequence files with protocol files to generate a synthesis run. Key features on this screen include:

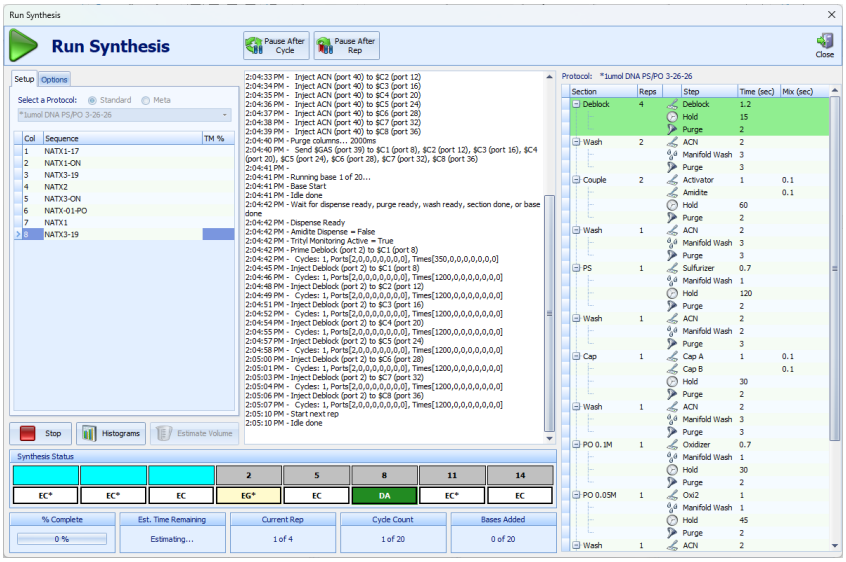


Figure 5-9: Run Synthesis Screen

- Pause After Cycle — Pauses the run at the end of the current cycle
- Pause After Rep — Pauses at the end of the current section repetition
- Stop — Pauses the run immediately
- Resume — Resumes synthesis after a pause
- Cancel — Cancels the synthesis immediately
- Start at Base X — Starts all columns at a specified base (must be set before starting)
- Pause Before Base X — Pauses before the selected base cycle (can be set during a run)
- Pause After Time — Pauses before the next cycle after a user-set time period HH:mm (can be set during a run)
- Estimate Volume — Calculates estimated reagent consumption before starting
- Color Display — Shows which amidites are being used in the current cycle and column types required
- Protocol Table — Live feed of the current cycle and synthesis step
- TM % — Displays the current estimated yield via trityl waste measurement

The status panel in the middle of the screen displays: software version, sequence names, protocols used, current base cycle, current rep, dispense information, trityl readings, any pauses, and a timestamp of each action. At the bottom: percent complete, time estimate, current base, current repetition, cycle count, and bases added.

IMPORTANT

Users can terminate a specific column's sequence mid-run by holding Ctrl and left-clicking the corresponding well in the Color Display.

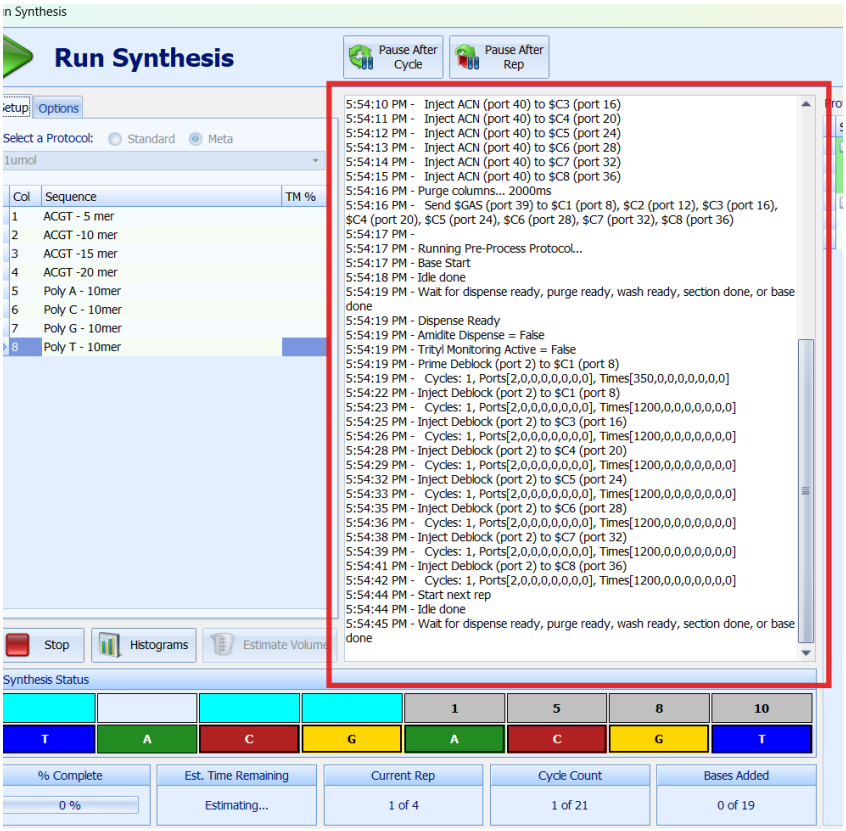


Figure 5-10: Run Synthesis status display

5.6.2 Estimate Volume

The Estimate Volume function calculates estimated reagent and amidite consumption for a planned run before starting synthesis. Running a volume estimate before every synthesis is strongly recommended.

To use: Load your sequence and protocol, select Estimate Volume before clicking Start, review the consumption figures, and confirm sufficient volume is available in each bottle.

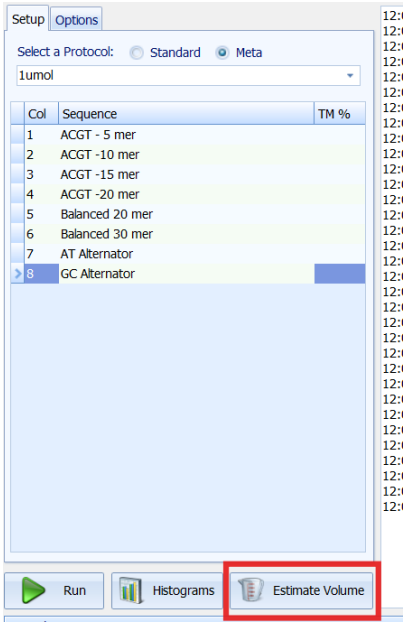


Figure 5-11: Estimate Volume Dialog

IMPORTANT

The Estimate Volume function requires that flow rates have been calibrated in the Flow Rates screen. See Section 5.5.2 — Flow Rates for more information.

5.7 Manual Control

⚠ CAUTION

Manual Control bypasses normal protocol logic and directly actuates instrument valves. It should only be used by trained users during maintenance, troubleshooting, or shutdown procedures.

The Manual Control screen provides direct access to every valve on the instrument. It is used during the shutdown procedure to clear lines, during troubleshooting to verify flow on individual valves, and during routine maintenance tasks.

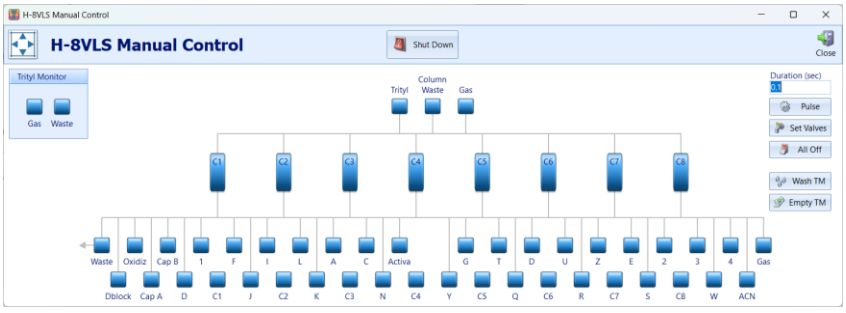


Figure 5-12: Manual Control Screen

Function	Description
Duration (sec)	Sets the duration in seconds for the Pulse function.
Pulse	Fires the selected valve(s) for the entered duration, then closes automatically.
Set Valves	Opens the selected valve(s) and holds them open until All Off is pressed.
All Off	Closes all valves immediately.
Wash TM	Sends ACN through the trityl monitor to wash the trityl vials.
Empty TM	Sends argon gas through the trityl monitor to purge remaining liquid.
Shut Down	Launches the guided shutdown procedure.

5.8 Data Management — Backup/Restore

The Backup/Restore screen (Setup/Utilities tab) allows users to save a complete backup of the K&A database or restore from a previously saved backup. A backup captures: Sequences, Protocols, Port Assignments, Reagents/Amidites, Backbone Modifications, and Configuration settings.

Figure 5-13: Backup/Restore Screen

IMPORTANT

Sierra BioSystems recommends backing up the database at a minimum of once per quarter, and any time significant changes are made. Save backup files to an external drive or network location, not the instrument computer itself.

⚠ WARNING

Restoring from a backup will completely overwrite the current database. This action cannot be undone. Ensure you are restoring the correct backup file before proceeding.

6. Operation

6.1 Typical Workflow

A typical synthesis run follows the workflow below:

1. Configure reagents and port assignments if necessary
2. Create or select synthesis protocol
3. Enter or import sequences
4. Prime reagent and amidite lines
5. Run Estimate Volume
6. Start synthesis
7. Monitor TM yield during the run

6.2 Preparing the Instrument

Before beginning any synthesis run, confirm the following:

- Gas supplies are connected and within operating pressure range
- All tubing and fittings are secure with no visible leaks
- Waste container is connected and not full
- Reagent and amidite bottles are installed and properly pressurized
- System is powered on and software is running
- The software is communicating with the instrument (device status indicators are active)

> **IMPORTANT**
> Ensure all reagent bottles are properly pressurized before starting a run. Improper pressure will result in poor delivery and failed synthesis.

6.3 Running a Synthesis

Step-by-Step Procedure

1. Verify reagent and port assignments.
2. Confirm reagent bottles are pressurized.
3. Import or define sequences in the Sequences screen.
4. Navigate to the Priming screen and prime the system. 350 ms minimum is recommended as a starting value for each line. Use Auto Prime to prime all lines sequentially.
5. Navigate to the Synthesis tab → Run Synthesis.
6. Load the sequence file into the column positions.

7. Select the appropriate protocol (Standard or Meta).
8. Load synthesis columns into the instrument.
9. Use Estimate Volume to confirm sufficient reagent quantities are available. Visually inspect bottles to verify sufficient reagent.
10. Select any additional options: Start at Base, Pause Before Base, and Pause After Time.
11. Click Run to begin synthesis.

Figure 6-1: Synthesis columns loaded in instrument

> **NOTE**
> If some column positions are not in use, install bypass connectors in those positions. This ensures any misdirected reagent flows to waste rather than an open port.

6.4 Monitoring the Run

During the run, monitor system performance to ensure proper operation. Watch for: system status in software, pressure stability, trityl values (if enabled), and reagent levels.

> **⚠ CAUTION**
> Do not allow reagent bottles to run dry during a synthesis as that can be detrimental to results.

6.4.1 System Status

The middle screen displays the status of actions the instrument is undertaking. This information is also stored in the log file. The status screen includes: software version, sequence names, protocols used, current base cycle, current rep, dispense information, trityl readings, any pauses, and a timestamp of each action.

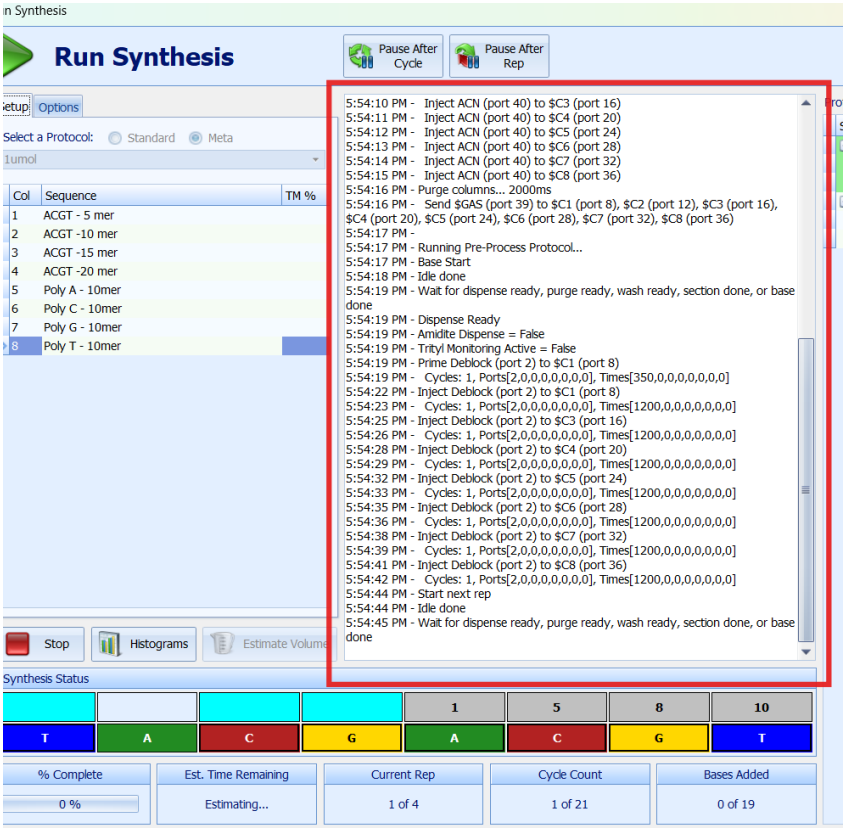


Figure 6-2: Run Synthesis status display

> **IMPORTANT**
>
> The time estimate is approximate. It functions by timing the previous cycle and multiplying by the number of cycles remaining. It will not be 100% accurate if there is variance in cycle times.

6.4.2 Pausing a Run

Option	Description
Pause After Cycle	Pauses the run after the current cycle is complete
Pause After Rep	Pauses after the current repetition
Pause Immediately	Pauses the synthesis immediately
Resume	Resumes synthesis after a stop or pause
Cancel	Cancels the synthesis immediately

Start at Base X	Starts all columns at the base indicated (must be set before starting)
Pause Before Base X	Pauses the software before the selected base cycle
Pause After Time	Pauses before the next cycle after a set period (HH:mm)

If pausing to refill bottles: Pause the run, refill reagents, allow the instrument to reach its usual pressure level, prime affected lines in the Priming Screen, then resume the run.

> **NOTE**
> Pauses are recommended between cycles to avoid leaving columns exposed to reagents for too long, which may be detrimental to synthesis quality.

6.5 Trityl Monitoring

> **IMPORTANT**
> Trityl monitoring provides a qualitative indication of coupling efficiency during synthesis. It does not represent final purity, identity, or absolute yield. All synthesized oligonucleotides should be verified using appropriate analytical QC methods (e.g., HPLC, LC-MS). Trityl data should not be relied upon as the sole indicator of product quality.

The trityl monitor is a photometer that measures trityl levels in the vial for each column individually using 470 nm light. The Trityl Histograms can be accessed by selecting the Histograms button in the Run Synthesis Screen.

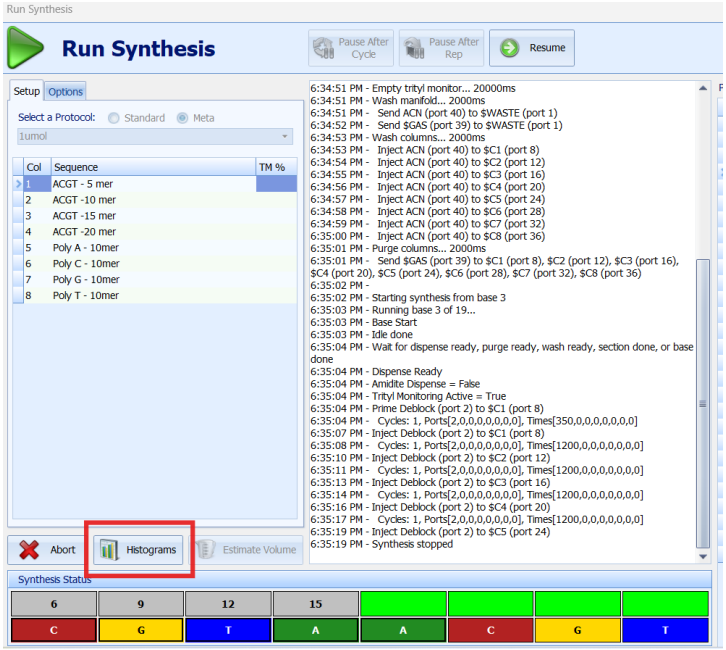
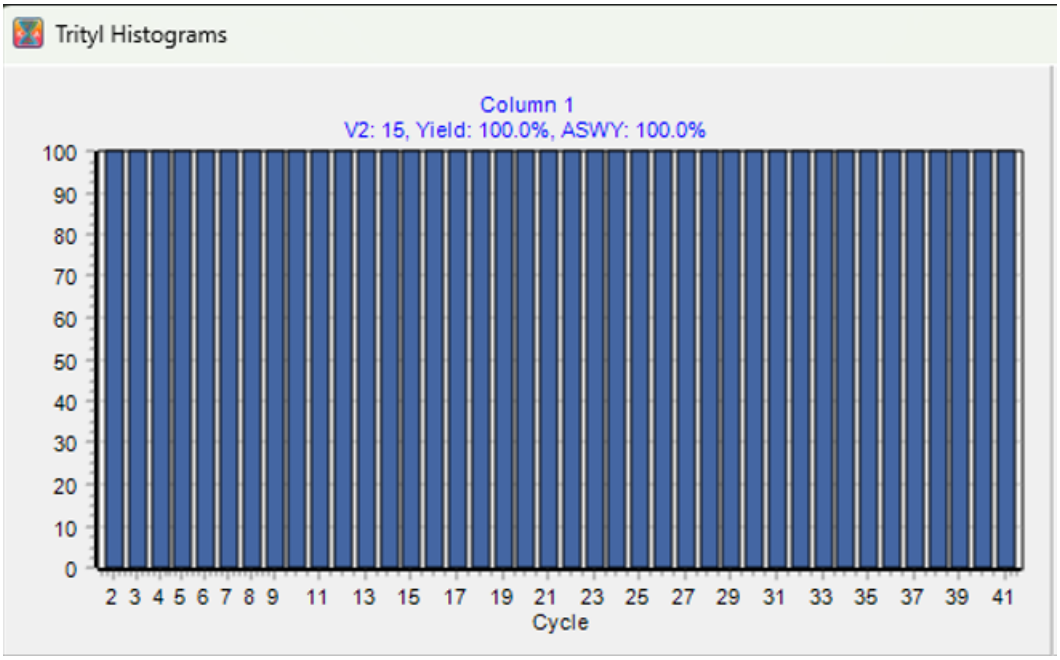


Figure 6-3: Trityl Histogram display

> **NOTE**
> The histograms will only populate after the second detritylation step (V2).

V2 Value

V2 represents the transmission measurement taken during the second trityl collection of the synthesis. This value serves as the reference for all subsequent trityl measurements. Values range from approximately 1 (very strong color, high coupling efficiency) to 4095 (full transmission, no color).



> **IMPORTANT**
> The accuracy of V2 is critical. If V2 is set incorrectly due to a poor first coupling, all subsequent yield calculations will be offset. Acceptable V2 values typically fall between 1 and 100, though this varies by chemistry and dilution conditions.

Yield Calculations

Total yield is calculated using the following formula:

[1 – (LastTritylRead – TritylBaseline) / (4095 – TritylBaseline)] × 100

Where:

TritylBaseline = the V2 reference value

LastTritylRead = transmission at the current cycle

4095 = maximum possible sensor reading

ASWY represents the average coupling efficiency per cycle:

ASWY = [Total Yield ^ (1 / Number of Cycles)] × 100

An ASWY of 97–99% per step is typical for well-optimized synthesis conditions.

6.6 Completing a Run

After synthesis completes:

- Remove completed columns from the instrument.
- Verify all positions are finished.
- Proceed to post-processing or next run.

> **NOTE**
> Some positions may complete earlier than others. You may remove completed columns while the system continues running remaining positions. Be sure to place a bypass connector in place of any removed columns.

6.7 Shutdown Procedure

The software guides the user through the full shutdown sequence automatically. All valve actuation and line clearing steps are handled by the software — the user only needs to follow the on-screen prompts.

The Shutdown procedure is recommended in the following scenarios:

- **End of use** —run the shutdown procedure when synthesis is complete before leaving the instrument unattended for an extended period
- **Extended idle periods** — if the instrument is not used for several days or longer
- **Before any maintenance** — membrane replacement, valve replacement, bottle cap changes, or any procedure requiring opening the instrument or disconnecting lines

- **Before moving the instrument**
- **Any time a leak or pressure issue is suspected**

> **⚠ WARNING**
> Never turn off the high-pressure supply while reagent or amidite bottles are still pressurized. The shutdown procedure will prompt you to depressurize bottles at the correct point. Do not close the high-pressure gas switch before this prompt appears.

Figure 6-4: Manual Control — Shutdown button

1. Open the Manual Control screen from the Synthesis tab.
2. Click the Shut Down button at the top of the screen.
3. A confirmation dialog will appear. Click Yes to begin.
4. The software will begin the shutdown sequence. Active valves are highlighted in green in the valve grid.
5. At certain steps the software will pause and display a prompt asking the user to perform a manual action. Read each prompt carefully, perform the requested action, then click OK to continue.
6. When prompted, turn off **only the bottle pressures** while keeping the main argon and compressor pressure valves untouched.
7. Depressurize the bottles by unthreading the bottle caps from all connected reagents and amidites. The argon pressure gauge should read zero or close to zero.
8. Continue following each prompt until the shutdown sequence is complete.
9. You may now power down the instrument or computer if necessary.

> **⚠ WARNING**
> Failure to depressurize all bottles will lead to cross-contamination of reagents/amidites.

> **NOTE**

Do not close the software or power down the instrument until the shutdown sequence has fully completed. Interrupting the sequence mid-way may leave reagent in the lines.

Long-Term Idle

If the system will not be used for an extended period:

- Ensure all lines are dry
- Perform the Shutdown procedure
- Remove sensitive reagents if necessary
- Store the system in a stable environment within the specified temperature range

7. Post-Processing

7.1 General Workflow

Post-processing procedures vary depending on the oligonucleotide chemistry, protecting groups, and purification strategy used. Users should confirm each step against their specific chemistry protocols and consult Sierra BioSystems support or their reagent supplier for guidance on non-standard applications.

The synthesizer produces a crude oligonucleotide attached to solid support. Post-processing removes the oligonucleotide from the support, removes protecting groups, and purifies the product prior to downstream use.

1. Step 1 — Cleavage from Solid Support: Transfer the column contents or CPG to an appropriate vessel. Add the cleavage reagent appropriate for your chemistry and incubate for the recommended time and temperature.

> **NOTE**
> Cleavage conditions vary by chemistry. Standard DNA typically requires 1–2 hours at 55–65°C in concentrated ammonium hydroxide. RNA and modified oligos may require different conditions. Always confirm with your amidite supplier's recommendations.

2. Step 2 — Deprotection: If cleavage and deprotection are not performed simultaneously, complete deprotection using the appropriate reagent and incubation conditions.
3. Step 3 — Transfer and Drying: Transfer the solution to a clean collection tube. Evaporate using a vacuum centrifuge (SpeedVac) or gentle nitrogen stream. Avoid excessive heat during drying.
4. Step 4 — Resuspension: Resuspend the dried crude oligonucleotide in nuclease-free water or TE buffer at the desired concentration.
5. Step 5 — Desalting and/or Purification:
    a. Cartridge desalting (C18 or OPC) — Suitable for most standard oligonucleotides
    b. HPLC purification — Recommended for DMT-On oligos, modified oligos, or high-purity applications
    c. PAGE — Used when HPLC is not available, particularly for longer oligos
6. Step 6 — QC Analysis: Verify identity and purity by UV absorbance (260 nm OD measurement), mass spectrometry (ESI-MS or MALDI), or analytical HPLC.

7.2 Safety

> **⚠ WARNING**

Cleavage and deprotection reagents — including concentrated ammonium hydroxide, methylamine, and other volatile or corrosive chemicals — are hazardous. Perform all post-processing steps in a fume hood with full PPE.

⚠ CAUTION

Pressurized vessels may build significant internal pressure during heated deprotection steps. Allow vessels to cool to room temperature in a fume hood before opening.

8. Troubleshooting

8.1 Common Issues

The table below covers common issues and initial steps. For detailed maintenance procedures such as membrane replacement, valve replacement, and compressor servicing, refer to the K&A Labs Service Manual.

Issue	Likely Cause	Initial Action
No flow from a line	Tubing blockage, membrane issue, valve issue, crimped gas line, or insufficient pressure	Check gas and liquid tubing for kinks. Ensure argon and compressor pressures are within operational range. Contact service if issue persists.
Low or inconsistent pressure	Gas leak, loose fitting or bottle cap, misfiring valve	Inspect all bottle caps and fittings. Check gas supply pressure. See Section 8.2 for pressure leak identification.
Poor yield / low ASWY	Chemistry issue, poor delivery, or incorrect V2	Check reagent quality. Verify mid-synthesis priming is enabled. Review trityl histogram. Contact support if unresolved.
Estimate Volume not populated	Flow rates have not been calibrated	Calibrate flow rates in the Flow Rates screen (Section 5.5.2).
Software not communicating	Ethernet disconnected or incorrect settings	Verify instrument is receiving power. Check Ethernet cable. Verify Ethernet Card IP address is 192.168.1.1. Verify power settings are set to never sleep.
First column yield lower than others	Dead volume in pathway or ACN diluting initial deblock	Enable Mid-Synthesis Priming in Configuration. Ensure thorough priming before run.

8.2 Identifying a Gas Pressure Leak

Signs of a leak include:

- Fast pressure drop when Argon is cut off (>.01 MPa in 1 minute)
- Visible liquid accumulation on the main block
- Air bubbles observed in reagent lines
- Irregular or reduced reagent delivery
- Trityl signals inconsistent across columns

Initial inspection steps:

1. Inspect all luer-lock fittings and tubing connections.
2. Verify bottle caps are fully tightened and O-rings intact.

3. Run a Flow Rate Check to confirm proper fluid delivery.
4. Confirm pressure regulators maintain stable readings.

> **NOTE**
> For guidance on membrane replacement, valve replacement, and detailed leak diagnostics, refer to the K&A Labs Service Manual.

8.3 Trityl Monitor — High V2 Values

High V2 values indicate low trityl color intensity during the second detritylation measurement. Possible causes include:

- Poor initial coupling
- Degraded amidites
- Incomplete priming of reagent lines prior to synthesis
- Mid-synthesis priming not enabled
- Incorrect deblock reagent concentration

Actions:

- Confirm proper priming of amidite lines
- Verify reagent freshness and storage conditions
- Check column support type
- Review synthesis protocol parameters
- Always further confirm results with additional QC steps such as HPLC or yield analysis

8.4 When to Contact Support

Contact Sierra BioSystems support if you experience:

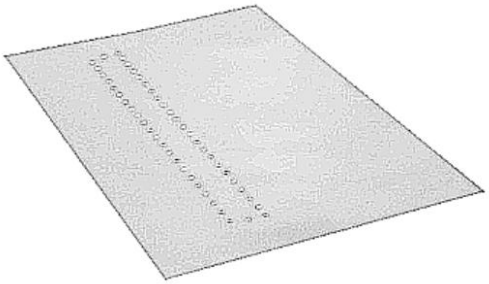
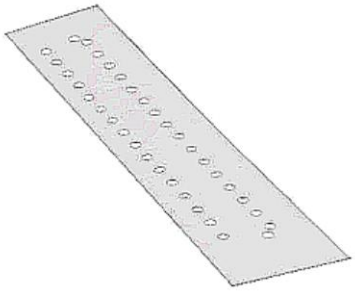
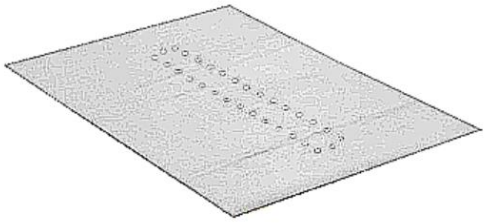
- Repeated failures that cannot be resolved by the steps above
- Electrical issues or error messages related to the valve controller or pressure sensor
- Unresolved pressure issues that do not resolve after inspecting fittings and tubing
- Any hardware damage or suspected component failure

Contact Method	Details
Support Center	https://support.sierrabio.com
Email	support@sierrabio.com
Phone	+1-209-396-1969

9. Parts & Ordering

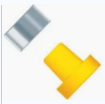
Contact Sierra BioSystems to order replacement consumables and spare parts. Below is a table of common components that might need replacement and recommended quantities.

Membranes

Part	Standard SKU	Spare Qty	Image
40-Port Membrane (H8SE / H16 / H28 / H32)	11-8003	2	
24-Port Membrane (H8 / H6 / H4)	11-8012	2	
5x3 Port Membrane (Waste Manifolds)	11-8004	4	

Tubing & Fittings

Part	Standard SKU	Spare Qty	Image
FEP Tubing, 1/16" OD × 0.030" ID	12-0007	20 ft (~6 m)	

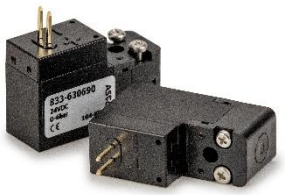
Super Flangeless Nut Headless, 1/16" OD	10-0222	10	
Super Flangeless Ferrule with SST Ring, 1/16" OD	10-0144	10	
Luer Fitting, 1/4"-28 UNF Female (top)	10-8016	8	
Luer Fitting, 1/4"-28 UNF Male (bottom)	10-8015	8	
Tefzel (ETFE) Plug, 1/4-28	10-0228	10	

Bottle Caps & Daisy Chains

Part	Standard SKU	VLS SKU	Spare Qty	Image
20-400 Bottle Cap	03-0041	03-0042	1	
28-400 Bottle Cap	03-0044	03-0045	1	

GL38 Bottle Cap	03-0050	03-0051	1	
GL45 Bottle Cap	03-0052	03-0053	1	
GL38 Daisy Chain	03-0073	—	1	—
GL45 Daisy Chain	03-0074	—	1	—

Valves & Filters

Part	Standard SKU	Spare QTY	Image
Pneumatic Valve (Micro 10, 3/2-way)	13-8001	10	
Particle Filter, 10 µm, for 1/16" OD Tube	22-0037	40	

Syringe Column Adapters

Part	Standard SKU	Spare Qty	Image
1 mL Syringe Column Adapter	15-8057	As needed	
3 mL Syringe Column Adapter	15-8058	As needed	
6 mL Syringe Column Adapter	15-8059	As needed	
12 mL Syringe Column Adapter	15-8055	As needed	
20 mL Syringe Column Adapter	15-8056	As needed	

For purchasing inquiries, availability, or pricing, contact:

- Email: sales@sierrabio.com or info@ka-labs.de
- Support Center: https://support.sierrabio.com

10. Support & Service

10.1 Technical Support

If you experience issues that cannot be resolved using the troubleshooting procedures in this manual, Sierra BioSystems technical support is available to assist.

Before contacting support, please gather the following information if possible:

- Instrument model
- Instrument serial number
- Software version
- Description of the issue
- Relevant synthesis log files or screenshots
- Recent changes to reagents, protocols, or hardware

10.2 Contact Information

Contact Type	Details
Support Center	https://support.sierrabio.com
Technical Support Email	support@sierrabio.com
General Inquiries & Parts	sales@sierrabio.com or info@ka-labs.de
Phone	+1-209-396-1969
Sierra BioSystems Address	21085 Longeway RoadSonora, CA 95370, United States
K&A Labs GmbH Address	Industriering 6, 64850 Schaafheim, Germany

10.3 Software Logs and Diagnostic Data

Many issues can be diagnosed using synthesis log files generated by the KA Labs software. When contacting support, users may be asked to provide: synthesis log files, flow rate data, screenshots from the Run Synthesis screen, and exported protocol or sequence files. These files can typically be exported directly from the KA Labs software.

10.4 Warranty

10.4.1 Limited Warranty

Sierra BioSystems warrants that K&A DNA Synthesizer instruments are free from defects in materials and workmanship under normal use and operation for a period of one (1) year. The warranty period begins on the date of installation, provided installation is performed by a K&A or Sierra BioSystems-certified installer.

10.4.2 What Is Covered

- Defects in electronics, materials, and workmanship
- Replacement parts required to resolve valid warranty issues
- Labor required to diagnose and repair the instrument
- Shipping costs associated with approved warranty repairs

10.4.3 What Is Not Covered

- Normal wear items or consumables: valves, bottle caps, tubing, seals and O-rings
- Cosmetic damage such as scratches or discoloration
- Damage resulting from misuse, improper operation, or unauthorized modification
- Damage caused by improper electrical supply, gas supply, or environmental conditions
- External components: compressor, computer, monitor

10.4.4 User Responsibilities

To maintain warranty coverage, users are expected to:

- Perform routine maintenance as described in the K&A Labs Service Manual
- Maintain clean and dry pneumatic air supply
- Periodically purge moisture from the compressor system
- Avoid leaving reagents or amidites in lines where crystallization may occur
- Perform reasonable troubleshooting steps when issues occur

For the most current warranty information, visit: https://support.sierrabio.com/ka-warranty

10.5 Service Contracts

After expiration of the standard warranty period, Sierra BioSystems offers service contracts to help maintain instrument reliability, minimize downtime, and provide continued technical support. Service contracts may include:

- Scheduled preventive maintenance visits
- Priority technical support from Sierra BioSystems engineers
- Discounted repair services and replacement parts
- Access to software updates and technical guidance

For information about available service plans, pricing, or contract eligibility, contact: sales@sierrabio.com or visit https://support.sierrabio.com/ka-service-contract

Appendix — Glossary

ACN (Acetonitrile) — A solvent used extensively in oligonucleotide synthesis for washing columns and clearing lines between reagent deliveries.

Amidite (Phosphoramidite) — The protected nucleoside building block used in automated oligonucleotide synthesis. Amidites are moisture-sensitive and must be stored and handled accordingly.

ASWY (Average Step-Wise Yield) — A calculated metric representing the average coupling efficiency per synthesis cycle. Formula: ASWY = [Total Yield ^ (1 / Number of Cycles)] × 100. A well-optimized synthesis typically achieves 97–99% ASWY.

Auto Prime — A priming mode in which the software sequentially fires each valve that has a time value entered, one at a time, for its set duration.

Backbone Modification — An alteration to the phosphate linkage between nucleotides. Common modifications include phosphorothioate and methylphosphonate linkages.

Bypass Connector — A small fitting used to occupy an unused column position, ensuring any misdirected reagent flows to waste.

Capping — A synthesis step that terminates any unreacted support-bound sequences. Capping reagents are typically Cap A (acetic anhydride) and Cap B (N-methylimidazole).

Column — The reaction vessel containing the solid support to which the growing oligonucleotide chain is attached.

CPG (Controlled Pore Glass) — A common solid support material used in oligonucleotide synthesis. The first nucleoside of the sequence is pre-loaded onto the CPG packed within the synthesis column.

Coupling — The chemical reaction in which an activated phosphoramidite is joined to the 5'-hydroxyl of the support-bound nucleoside chain, extending the oligonucleotide by one base. Coupling efficiency directly determines overall synthesis yield.

Dead Volume — The empty space occupying a reagent line or manifold before any delivery occurs. Must be cleared by priming before synthesis.

Deblock (Detritylation) — The step that removes the DMT protecting group from the 5'-hydroxyl, freeing it for the next coupling reaction.

DMT (4,4'-Dimethoxytrityl) — An acid-labile protecting group on the 5'-hydroxyl of each phosphoramidite. Removed during the deblock step of each cycle. DMT-On synthesis retains the final DMT group for purification purposes..

Estimate Volume — A software function that calculates estimated reagent and amidite consumption for a planned run before it begins.

Flow Rates — A calibration screen used to measure and record the actual volume delivered per unit time for each reagent and amidite line.

Hold to Prime — A priming mode in which the selected valve remains open for as long as the user holds the button. Used for visual confirmation of flow or clearing stubborn lines.

Luer Lock — A standardized threaded fitting system used to connect synthesis columns to the instrument's fluid lines. Provides a secure, leak-resistant seal that can be connected and removed without tools.

Manifold — The internal common channel within the valve block through which reagents flow after passing through the membrane.

Membrane — A flexible sealing element within the valve block that controls fluid flow. Replaced approximately every four months.

Meta Protocol — A composite synthesis protocol that links multiple Standard Protocols together to automate base-specific and sequence-step-specific handling.

Mid-Synthesis Priming — An optional feature that automatically primes a valve the first time it is used within each synthesis section.

Oligonucleotide (Oligo) — A short, single-stranded sequence of nucleotides synthesized on the K&A instrument.

Oxidizer (OXI) — A reagent used after each coupling step to convert the phosphite triester linkage into a stable phosphate triester. The default backbone reagent.

Phosphoramidite — See *Amidite*.

Port Assignments — A screen that defines which reagent or amidite is connected to each physical valve port on the instrument.

Priming — The process of pushing fresh reagent or amidite through a line to eliminate dead volume before it reaches the synthesis column.

Pulse Prime — A priming mode in which the selected valve(s) are fired once for the entered duration. Used for targeted, single-event priming of one or more specific lines.

Purge — A protocol step that opens the drain valves and applies gas pressure to push liquid out of the synthesis column, clearing it in preparation for the next reagent delivery.

Solid Support — The insoluble matrix (typically CPG or polymer beads) contained within the synthesis column, to which the first nucleoside is attached and upon which the oligonucleotide chain is built stepwise.

Standard Protocol — A synthesis cycle file defining the step-by-step sequence of reagent deliveries, holds, purges, and washes.

Sulfurizer — A reagent used as an alternative to oxidizer to produce a phosphorothioate backbone.

TCA (Trichloroacetic Acid) — A commonly used deblocking reagent that removes the DMT protecting group during the deblock step. Also referred to as deblock solution.

Trityl Monitor — An optical photometer that measures the absorbance of the orange trityl cation released during each deblock step at 470 nm.

Universal Support — A solid support not pre-loaded with a specific nucleoside, allowing any 3' base to be incorporated as the first coupling step. Selected using the Univ checkbox in the Sequences screen.

Waste Container — The external vessel connected to the instrument's waste manifold lines that collects all waste solvents and reagents expelled during synthesis, priming, and shutdown. Must be monitored regularly and disposed of in accordance with applicable chemical waste regulations.

Waste Manifold — The network of tubing and fittings that routes all waste fluid away from the synthesis columns and trityl monitor to the external waste container.

V2 — The trityl transmission measurement taken during the second trityl collection of the synthesis. Serves as the baseline reference value for all subsequent yield calculations.