Enabling a Healthier World

Lonza

PyroTec® PRO Robotic Solution

FAQ Tech Tips

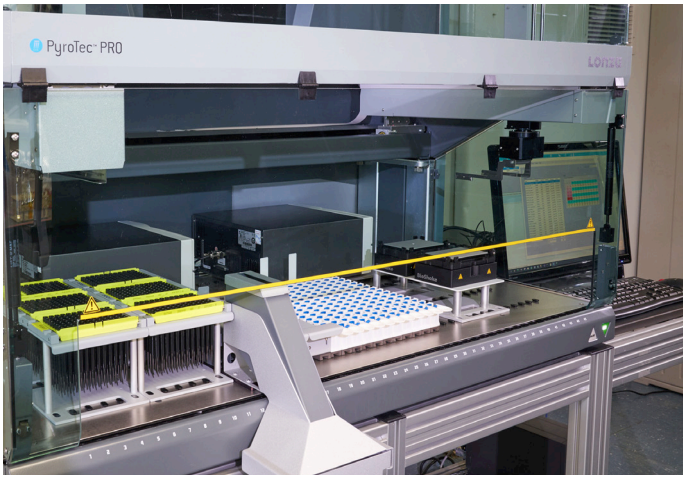
Move from manual benchtop processes to an automated workflow

The PyroTec® PRO Robotic Solution is a new automated, plate-based system that offers users the ability to move away from manual bench top processes associated with endotoxin testing. The PyroTec® PRO System is directly integrated with WinKQCL® Version 6 Software, achieving high-throughput sampling in three simple steps, offering a fully automated workflow for higher volume QC labs.

This Tech Tip will help users gain insight into assay automation and how it can transform a busy manual-based endotoxin laboratory into a streamlined, automated system. Topics include: sample dilution, integration of laboratory information management systems (LIMS), automation process workflow with WinKQCL® Software, deck setup and assay runs including technical specifications.

What is assay automation?

Assay automation is a custom solution comprised of software, hardware, integration and engineering to reduce or eliminate manual intervention in the performance of an assay. This requires a comprehensive look at your laboratory environment, an analysis of strengths, weaknesses, specific pain points, and a determination of how automation can be introduced to streamline and improve performance. Through process optimization, automation can result in time savings and error reduction.



Are there any consumables that need to be purchased to run the PyroTec® PRO Robotic Solution other than standard reagents, dilution tubes, and microplates?

Yes, specific pipette tips (P/N 0000229884) and troughs (P/N 0000229888) for the robotic system are purchased from Lonza.

Are all the consumables endotoxin-free?

All consumables, including pipette tips (P/N 0000229884), troughs (P/N 0000229888), 13 x 100 mm tubes (P/N N207), and microplates (P/N 25-340) used in the system are certified to be endotoxin-free (<0.005 EU/mL).

What are the power requirements?

- 100-240 Volts
- 50/60 MHz
- 1,200 VA

How much space is required for the PyroTec® PRO System?

The robot is 145 cm wide, 78 cm deep and 87 cm tall. A minimum of 10 cm around the instrument is required for proper function.

Depending on the configuration, the weight of the PyroTec® PRO System ranges from 187-200 kg.

Tables are available for purchase from Lonza that will accommodate the PyroTec® PRO System size and weight.

Can you provide an overview of product specifications for the PyroTec® PRO Robotic Solution?

Description	Answer
Throughput per run	42 (21 per plate in duplicate w/ duplicate PPC) with five point standard curve with two negative controls.
Sample handling	Yes
Prepares sample dilutions	Yes, 128 (includes sample tubes). All 128 tubes are available to make simple to complex dilution schemes.
Prepares auxiliary dilutions	Yes, for dilutions requiring other than liquid reagent water, i.e. Beta-Glucan blocker.
Reconstitution of RSE/CSE	No
Vortex of reconstituted RSE/CSE	No
Prepares endotoxin standards	Yes, flexibility to make up to eight standards per assay (most common is five).
Prepares positive product controls	Yes
Prepares negative controls	Yes
Incubates plate	Yes
Reconstitution of LAL reagents	Yes
Pipettes LAL to plate	Yes
Places plate in incubator	Yes
Barcode scanner	2D barcode; various standard formats supported. Barcodes are entered on the template in WinKQCL® Endotoxin Detection and Analysis Software as well as adaptability on the robot deck.
Sample tubes type	Pyrogen-free USP Type 1, 13 x 100 mm tubes with max volume of 8 mL per tube (128 tubes available)
Ancillary accessories	Tips, reagent reservoirs, and microplates supplied by Lonza certified down to 0.005 EU/mL
Deck setup time	Fully loaded plate: 5 minutes minimum.
Preparation of plate	Fully loaded plate: 5 minutes minimum.
Runtime in reader	Approximately 1 hour
Total time to result including preparation and run	1 hour 50 minutes for 21 samples tested neat
Sample test volume	100 µL; Minimum sample volume – 600 µL for samples to be diluted; 800 µL for samples to be tested neat
Method supported	**Kinetic chromogenic** and kinetic turbidimetric. rFC coming soon

Which type of liquid handling arm does the PyroTec® PRO System use, liquid or air driven?

The air LiHa™ technology forms a core element of Lonza's automated solution for endotoxin testing. The air LiHa™ uses air displacement technology to offer increased flexibility and greater convenience for a variety of applications requiring disposable tips. The air LiHa™ has a number of advantages for this application:

- A broad despensing volume ranging from 10 µL – 800 µL per channel
- A low level tip ejector that allows removal of disposable tips without risk of cross-contamination due to generation of aerosols
- Utilizes cLLD-capacitive liquid level detection – for fast liquid level detection and preferential non-contact dispensing, for optimum pipetting performance
- Offers fast dispense down to 10 µL with CVs below 6%
- Each channel is protected by an inline filter

WinKQCL® Endotoxin Detection and Data Analysis Software and Integration

How are manual steps controlled by software?

Pop-up windows in the WinKQCL® Software application guide the user through manual preparation (endotoxin, reagents, sample), and every step has to be actively confirmed by the user, which acts as a fail-safe. For data integrity purposes, it reduces the risk of operator error in setting up the deck.

Can manual and automated systems share the same WinKQCL® Software database for trending and data security?

Yes, the system is able to leverage the robust enterprise features of the WinKQCL® Software. This includes storing all information in a WinKQCL® Software database that can be shared amongst the organization.

The Software provides fine-grain user access controls and analytics, including trending across the historical records to assess excursions and utilize metadata to assist in root cause analysis investigations.

Can the PyroTec® PRO System interface with LIMS?

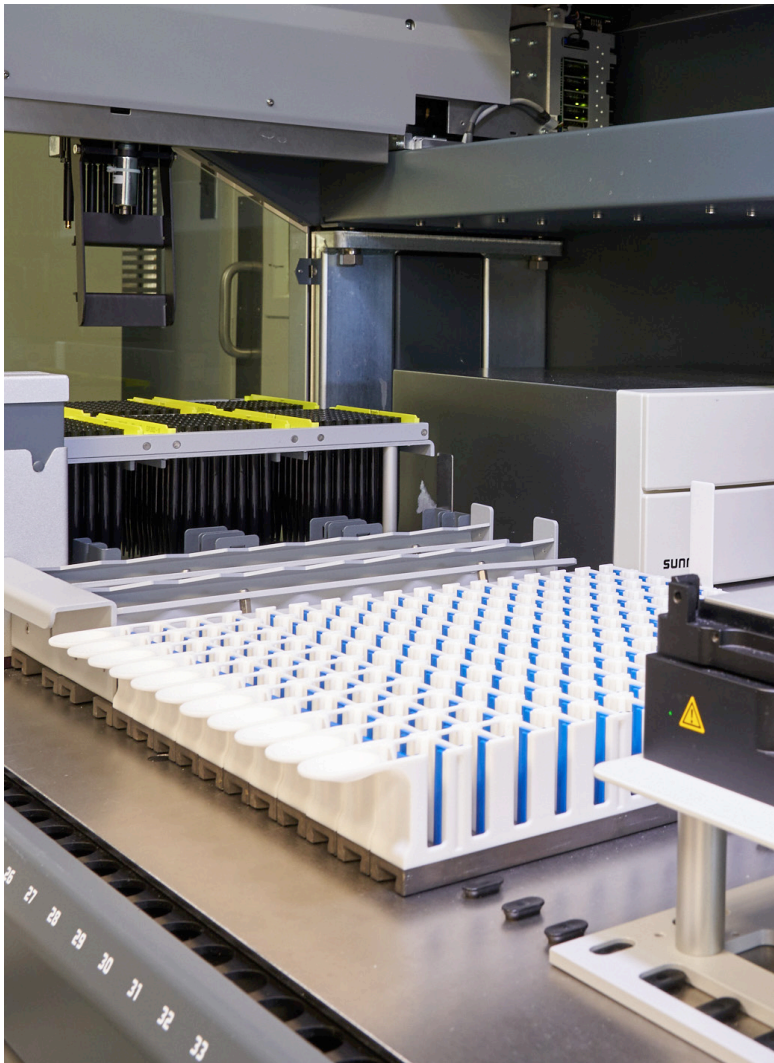
Yes, the end-user can setup and define the trigger for the data transfer. Lonza offers services to assist with this.

Can plate dilutions schemes be transferred to WinKQCL® Software if contained in a LIMS?

Yes, this information can be pulled from a LIMS if available. For example, an export created in CSV can be exported back into LIMS.

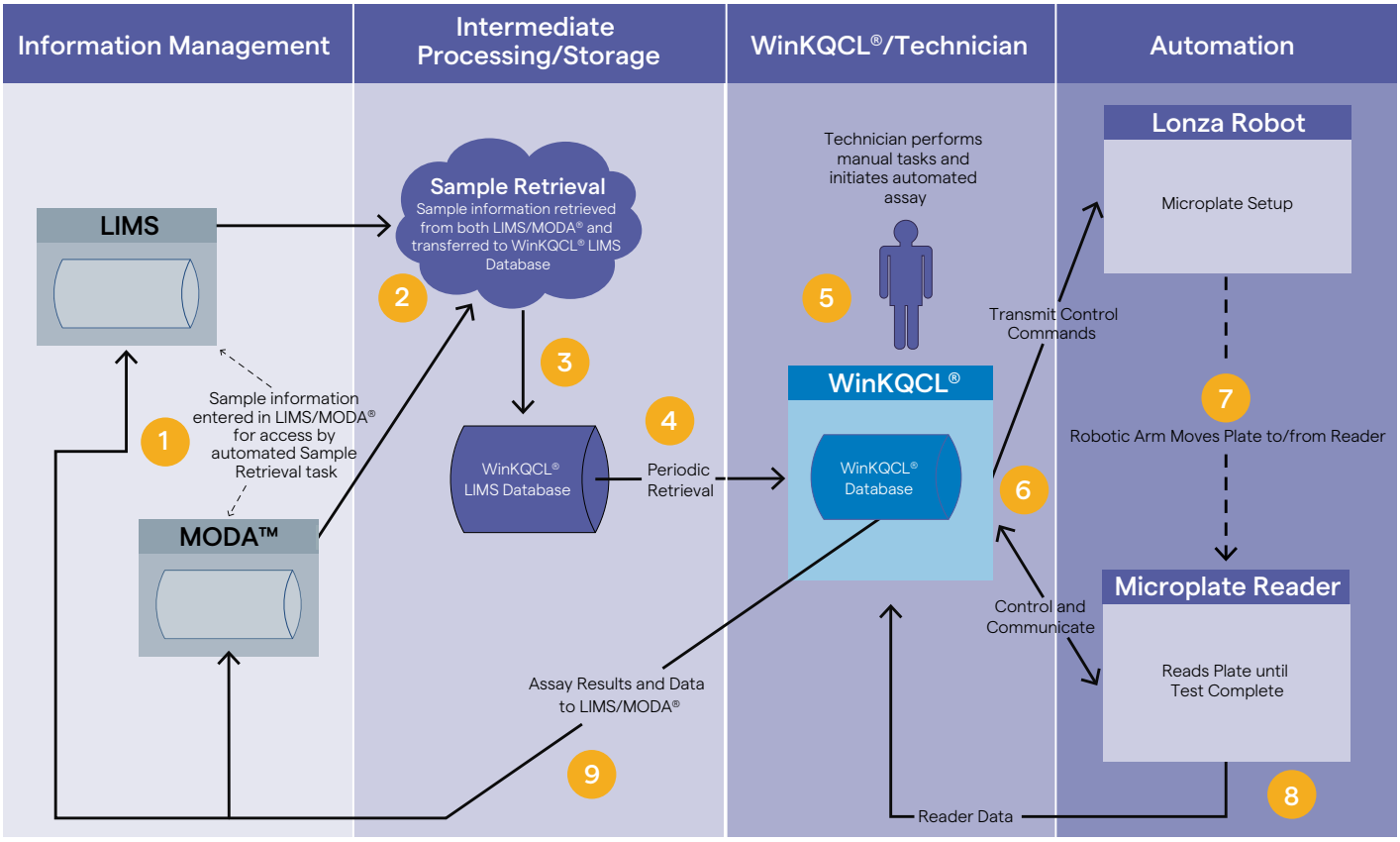
Is the PyroTec® PRO System compatible with other, non WinKQCL® Software?

The automation module providing dynamic scripting* and control of the robotic system is an integral part of Lonza's WinKQCL® Version 6 Endotoxin Analysis and Detection Software. It is not compatible with any other endotoxin detection software.

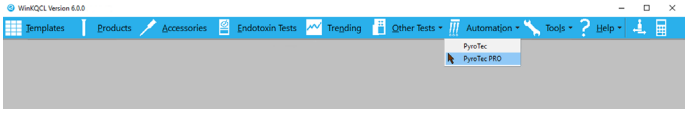


Endotoxin automation high-level process flow

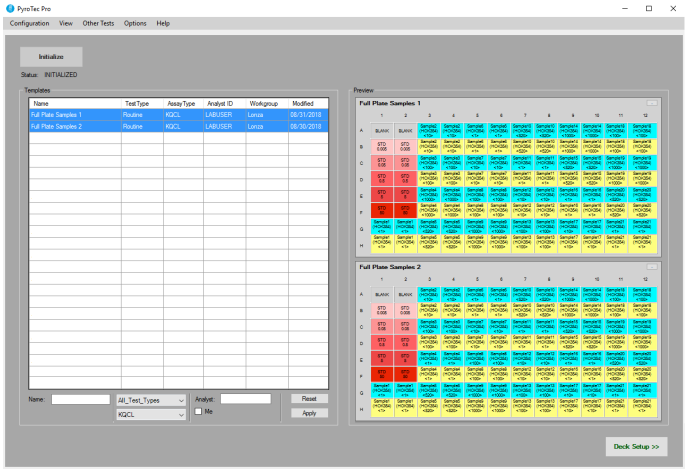
The endotoxin automation high-level process workflow on this page depicts the process in the automation module software, a fairly simple process. A User Manual will be included with the purchase of the PyroTec® PRO System with detailed instructions.



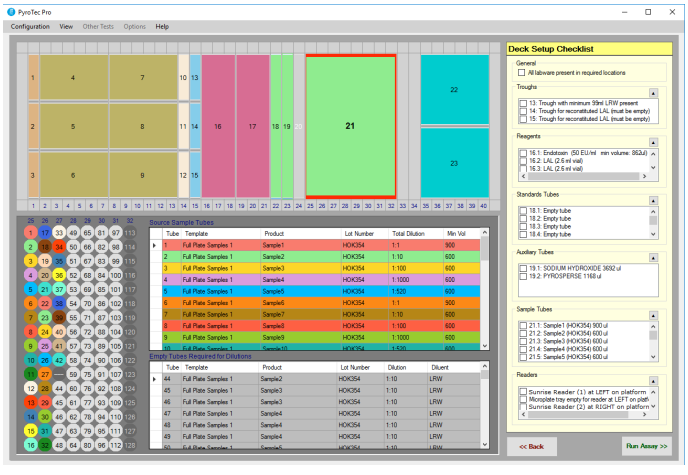
Step 1:
Initiate the automation module within WinKQCL® Software



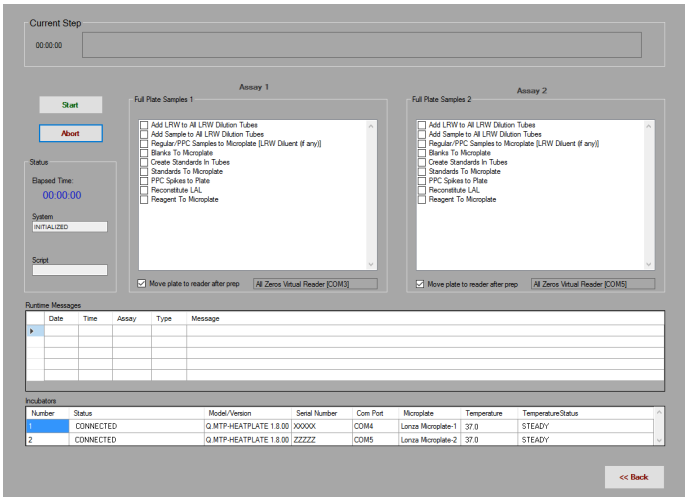
Step 2:
Select the template(s)



Step 3:
Setup the deck per the instructions provided



Step 4:
Initiate the automated run



Endotoxin
The endotoxin must be manually reconstituted and vortexed by the analyst – the same as would be done to perform a manual assay. After the analyst vortexes the endotoxin, the analyst must then place the endotoxin on the deck at the location specified in the deck layout instructions (Step 3).

Reagents
The robot reconstitutes the test reagents. The user is only required to place the open reagent vials at the locations specified in the deck setup instructions (Step 3).

Samples
Sample tubes containing the specified volumes must be placed at the locations specified by the deck setup instructions (Step 3). When barcode scanning functionality is implemented, the samples may be placed anywhere in the sample tube racks.

Deck Setup
The deck setup interface (shown in Step 3) provides detailed instructions regarding where components must be positioned, and what volumes of liquids must be present. Notice that there is a checklist to the right of the interface. The analyst must check off every item in the checklist to confirm that all positions and all volumes are correct. The software will not allow the automated assay to be run until all items in the checklist are confirmed.

Process

Is it possible to automate the standard dilutions?

The standard endotoxin dilutions are created by the system based on what is specified in the WinKQCL® Software Template specific for that assay. The PyroTec® PRO System can dynamically create the dilution scheme in the WinKQCL® Software.

The system does not reconstitute the stock endotoxin. The endotoxin must be reconstituted and vortexed offline by an analyst prior to running an automated assay – and then placed on the robot deck. The system uses the reconstituted endotoxin to create the standards.

Can you provide more information on the validation of endotoxin standards made using pipetting vs. vortexing?

The only manual step required is for the analyst to perform the initial reconstitution and vortex the initial stock CSE (or RSE) solution prior to running the assay. The robot prepares the standard curve. As we do not place a vortex mixer on the deck, the robot uses multiple aspirate/dispense steps to mix the standards, starting from the stock CSE solution. Study data confirm that this method is comparable to using a standard vortex. This method is included in the reagent package inserts for automation customers.

How does the PyroTec® PRO System mix samples after sample dilution?

All mixing on the system is done by the liquid handling robotic arm described above using similar multiple aspiration/dispense steps to those used in standard preparation.

How does liquid level detection work if different sample containers are used (conductive or pressure based?)

Conductive liquid detection is used by the PyroTec PRO® System. The system currently supports sample handling in 13 x 100 mm tubes. An option to allow use of commonly used 15 mL polystyrene sample tubes is planned for a future software release.

Can you run different samples (different treatment, dilution etc.) on one plate?

The system can accommodate a variety of samples on one plate with different dilutions applied to each sample. The system will apply whatever LAL reagent water dilutions are assigned to each sample on the WinKQCL® Software-generated endotoxin template. Each product (i.e. sample) defined in the WinKQCL® Software Database is configurable in the automation module software to use a specific liquid class, which determines the pipetting parameters to be applied (different liquid classes for aqueous, viscous, foamy, etc). When a new sample in the WinKQCL® Software Database is detected by the automation software, it is initially assigned a default liquid class, but a different liquid class can be assigned by the user if necessary. The automation software is also able to handle auxiliary dilutions such as beta glucan blocker (P/N N190) and Tris buffer(P/N S50-642). Auxiliary dilutions are configurable per sample. The order in which the auxiliary dilutions are to be applied also is configurable. In addition, the reagent water itself can be configured as an auxiliary dilution if necessary.

Am I limited in the amount and type of sample dilutions?

The user is limited only by the 48 spaces available for sample tubes on the deck layout. The user can create a multitude of sample dilutions with different sample types, utilizing LAL reagent water in addition to auxiliary dilutions simultaneously within the same run.

How many plates can be processed with the instrument?

The system can accommodate up to two independent microplates on a single run of the instrument (i.e. a single deck setup).

What are the limiting factors (reagents, consumables etc.)?

The limiting factor is deck space on the instrument. Currently the instrument has enough space to accommodate the labware required for two assays, including the ability to run two different types of assays e.g. one chromogenic and one turbidimetric assay, within a single run.

Is it possible to reload the instrument during a run with reagents, consumables, samples? Can you continuously load? Is an automatic retest supported?

After the last microplate is placed in the reader and control is transferred to the WinKQCL® Software to begin reading the plate, the instrument's work is complete. At that time, it would be possible to start preparing the deck for the next run. However, the next run should not be started until all microplate readers (either 1 or 2) have completed reading the microplates, and all microplates have been removed from the readers by the analyst. When two assays are performed in a single run, the assays are performed sequentially, not concurrently.

The instrument will not start the microplate setup for the second assay until the instrument has placed the microplate from the first assay in the reader to be read by the WinKQCL® Software. When the WinKQCL® Software begins reading the first microplate, the instrument will begin processing the microplate setup for the second assay. The system does not support an automatic retest of a failed sample. The analyst would need to add the sample to the next run to retest it.

Has any stability testing been performed for the endotoxin and lysate that stay on the instrument before use?

Our data indicate that the reagents should be placed on the deck prior to running the automated assay. This would be similar to the methodology when performing the assay on the bench. Customers should follow procedures similar to those in a manual assay, specifically, they would not allow reagents to sit on a benchtop for a long period of time prior to use. For the lysate specifically, it is not reconstituted until just prior to transfer to the microplate, so for any time spent on the instrument prior to that, the lysate is in powder form.

How long does the assay take, and can an assay be run overnight?

Typical runtime from start of the automated microplate set-up to completion of WinKQCL® Software reading the plate for a single assay is 1.5 hours – 2.5 hours, depending on the number of samples and the complexity of the dilutions. Therefore, a two-assay run will typically complete in 3 hours based on the simplest testing scheme. The system begins setup and preparation of the second assay while the first plate is being measured.

Because the PyroTec® PRO System is automated, once the assay is set up and the run initiated, the run can complete without human interaction, so it may run overnight or between shifts.

Can the PyroTec® PRO System be utilized with the PyroGene® rFC Assay?

The system currently supports both the Lonza Kinetic-QCL® Kinetic Chromogenic LAL Assay (for example, P/N 50-650H) and the PYROGENT® 5000 Kinetic Turbidimetric LAL Assay (for example, P/N N688). Support for the PyroGene® Recombinant Factor C Endpoint Fluorescent Assay (for example, P/N 50-658NV) is planned for an upcoming system release.

Service

How long does it take to perform the installation, operational and performance (IOPQ)?

There are many components that need to be installed and validated. Depending on the customizations needed, estimated time for installation and operational qualification is one week.

Can you provide more information on the IOPQ?

1. Installation Qualification (IQ) – ensures that the hardware and software are installed and configured properly in preparation for further testing. Basically, this verifies that the software is installed and that readers and incubators have been configured correctly.
2. Operational Qualification (OQ) – ensures that hardware and software components are in proper working order (confirms functionality of LiHa™ and RoMa arms and functionality to transfer microplates to/from readers).
3. Performance Qualification (PQ) – evaluates the overall functional performance of the system by performing actual automated endotoxin tests using Lonza endotoxin testing reagents. The technician runs an assay to challenge the repeat pipetting accuracy using a Lonza PQ routine test template with samples and dilutions.

What is involved in the annual Preventive Maintenance?

Annual Preventative Maintenance (PM) is included in the Te-Care™ service contract, offered through the Lonza-Tecan partnership. The PM includes general cleaning, greasing, adjustments and replacement of wear-and-tear parts. Any required updates for instrument reliability, usability and safety will be provided.

Note that Te-Care™ also provides access to Technical Support through Lonza's Scientific Support team, and on-site repairs.

What additional maintenance activities are required?

Routine maintenance tasks are detailed in the User Manual. They include daily inspection and cleaning of the DiTi cones and an inline filter test. Post use, the worktable should be cleaned and DiTi waste emptied. Weekly, a leak test should be performed to ensure tip accuracy.

Who should I contact for technical support?

Your first line of support is always Lonza Scientific Support (numbers are listed at the end of this document). Lonza's team of dedicated professionals will assess your issue and, if required, obtain further support from Lonza or Tecan Subject Matter Experts depending on your needs.

Our lab would benefit from utilizing the PyroTec® PRO Robotic Solution. What would we need to do prior to scheduling an install?

The best next step would to be to contact your sales representative to schedule a meeting to discuss:

- Sample throughput, workflows and types
- Site requirements
- Service and support
- Return on Investment.

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