3M Microbial Luminescence System (MLS)

MLS-USB Instrument User Guide

3M Microbial Luminescence System (MLS)

Manuel d’instructions de l’appareil MLS-USB Instrument

Sistema de luminiscencia pra detección microbiana 3M (MLS)

Guía de usuario del aparato MLS-USB

Sistema de Luminescência Microbiana (MLS) 3M

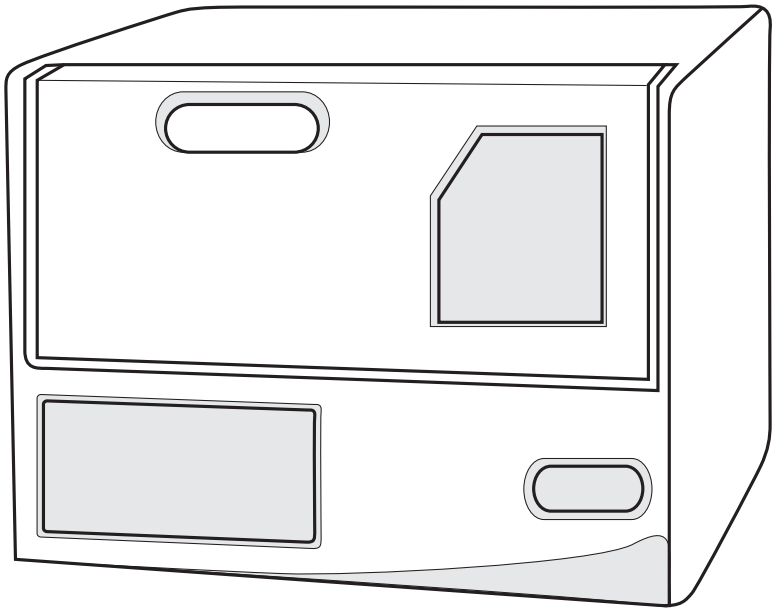
Manual do usuário do instrumento MLS-USB

3M 微生物ルミネセンスシステム（MLS）

MLS-USB装置ユーザーガイド

3M 微生物发光系统（MLS）

MLS-USB仪器使用者指南



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1. IMPORTANT INFORMATIONAL READING

Please read, understand, and follow all safety information contained in these instructions and the manufacturer's Operator's Manual prior to using the 3M™ Microbial Luminescence System (MLS-USB instrument). Retain these instructions for future reference.

1.1 Intended Use

The 3M MLS-USB is a microplate luminometer designed for microbial detection. Contact your official 3M Microbiology representative for a current listing of 3M MLS reagents intended for use with 3M MLS instruments. 3M has neither designed nor documented this instrument for use with other manufacturers' products. The unit has been designed and tested only to be used with the power cable that is supplied with the instrument. Only technicians that have been properly trained should operate the 3M MLS-USB instrument.

1.2 User Responsibility

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of this product do not constitute a guarantee of the quality of the matrices or processes tested.

1.3 Safety Information

Explanation of symbols:

	**ATTENTION** Consult Operator's Manual
	**WARNING** **To reduce the risk associated with hazardous voltage, which if not avoided, could result in death or serious injury:** • Periodically inspect the power cord for damage. Replace all damaged components. • Replace a damaged power cable only with a 3M designated power cable.
	**CAUTION** **To reduce the risk associated with environmental contamination, which if not avoided, may result in minor or moderate injury:** • Follow federal, state and local regulations when disposing of electrical components, including the in-line power supply. • This device incorporates parts or components that contain lead; it should be disposed according to local regulations and requirements for electrical and electronic devices of this nature.
	In compliance with European Directive 2002/96/EC of the European Parliament and of the Council, of 27 January 2003, on waste electrical and electronic equipment (WEEE), this product must not be disposed of as unsorted municipal waste. Instead this device must be collected separately in accordance with European local regulations. The solid bar used in conjunction with the rossed-out wheeled bin indicates that the product was put on the market after 13 August 2005. This symbol is applicable only in Europe.

1.4 Environmental Operating Conditions

Environmental Condition	Operating Condition	Units
Altitude	Not limited	meters
Normal Exhaust Temperature	Not applicable	°C
Max Exhaust Temperature	Not applicable	°C
Relative Humidity	Non-condensing at 37%	%
Voltage 50AN (US/Canada)	120 Volts	AC
Voltage 50AE (Europe)	240 Volts	AC
Voltage 50AJ (Japan)	100 Volts	AC
Frequency	50-60	Hertz
Pollution Degree	Emissions Class A (Harmonic Current Emissions and Voltage Fluctuations/Flicker: Class B)	

1.5 FCC Information

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide a reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the Installation and Use Guide, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case, the user will be required to correct the interference at his/her own expense.

1.6 Package Contents

- 3M MLS-USB Instrument
- Required MLS accessories
  - o Instrument power cable
  - o USB cable
  - o Syringes (3)
  - o Injectors (3)
  - o Tubing kit (for 3 injectors)
  - o Drain plate
- 3M MLS-USB Instrument User Manual
- Manufacturer's MLX Luminometer Operator's Manual
- Revelation™ software on CD
- Instrument Unpacking Instruction Sheet

1.7 User-Supplied Items

- Computer, connected through a USB port

  √ **Minimum requirements:** Windows-based PC, Pentium 500 MHz or higher, CD-ROM drive, ≥100 MB free space, ≥64 MB RAM (128 recommended), Microsoft® Windows 2000 or Windows XP
- Computer monitor (SVGA color graphics card and compatible color monitor)
- Mouse
- Keyboard
- Printer (optional; Windows 2000 or XP compatible)
- Pipettors capable of dispensing 50-1000 µL
- Pipette tips
- Incubator

2. OVERVIEW OF LUMINESCENCE

Adenosine triphosphate (ATP) is the basic energy molecule in all living organisms. It is present in all microbial, plant, and animal cells. The term 'somatic' cell is commonly used for plant and animal (non-microbial) cells. ATP can also be present in materials of organic origin as 'free' ATP (outside of cells) when cells are disrupted during natural processes or manufacturing processes, such as homogenization or heat treatment.

The term bioluminescence is used to describe a reaction that produces light through a biological process. In the presence of ATP, an enzyme called luciferase, together with its substrate D-luciferin (collectively referred to as L/L1 in this document) triggers a biochemical reaction that produces light as an end product. The amount of light emitted is proportional to the amount of ATP that is present. Low levels of light may be detected and measured using a sensitive luminometer.

Bioluminescence Reaction:

$$O_2 + \text{Luciferin} + Mg^{++} + \text{ATP} \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + CO_2 + \text{AMP} + \text{PPi} + \text{Light}$$

2.1 Bioluminescence and the 3M Microbial Luminescence System (MLS)

The 3M MLS platform utilizes ATP bioluminescence to detect microbial contamination in ultra-high temperature (UHT) or extended shelf-life (ESL) products. To obtain reliable results for microbial detection in these products, they must be incubated prior to testing, allowing microbial growth to a detectable level. Substantial reduction of somatic and free ATP is also critical to allow accurate detection of microbial (contaminant) ATP; this is achieved using an ATP-degrading enzyme called ATPase.

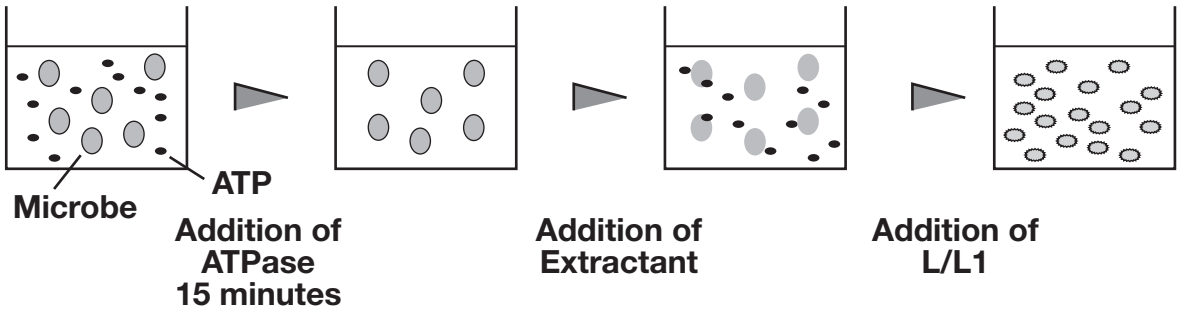
Since the amount of light emitted is directly proportional to the amount of ATP that is present, the bioluminescent reaction helps to determine the degree to which a test product is contaminated. If no microbes are present, little or no detectable ATP will remain after the ATPase treatment. Upon addition of L/L, the signal will be low or undetectable, and therefore within the 'Pass' limit.

Summary of a 3M MLS test assay:
Up to 96 samples may be loaded into an assay plate. The timing and addition of reagents is controlled by the MLS-USB instrument. **Step 1:** ATPase is added to each test well to degrade somatic and free ATP; followed by a 15-minute incubation. **Step 2:** an Extractant is added to each test well to lyse the intact microbial cells to release the microbial ATP. **Step 3**: the L/L reagent is added to the test well, creating a light-emitting reaction between the L/L and microbial ATP. The MLS-USB instrument reads the level of light that is emitted from each test sample and expresses the results in Relative Light Units (RLU). Figure 1 illustrates the steps in an assay using the 3M MLS.

Results are available in 40 minutes or less (depending on the number of test samples), compared to 2-5 days using traditional microbiological methods.

Default Pass/Fail criteria are established, however, these criteria may depend upon the type of product and user's requirements. Appropriate Pass/Fail criteria are initially established for each type of product by running multiple samples against existing standard methods.

Figure 1. Addition of ATPase, Extractant, and L/L to a Contaminated Milk Sample



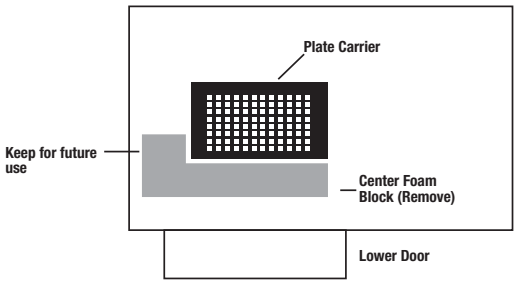
3. UNPACKING THE MLS-USB INSTRUMENT

It is important to start the installation process after you have decided on the placement of the MLS-USB instrument. The instrument is approximately 91 cm (36 in) wide, 61 cm (24 in), and 41 cm (16 in) deep.

1. Ensure that the location of the instrument allows easy access to the power outlet, and also allows the plate carrier to open without obstruction.

2. A gap of **at least 6** inches should be left around all sides to allow ventilation and prevent the instrument from overheating.
3. To unpack the instrument, remove the foam packing material from the top of the shipping carton. **CAUTION:** The MLS-USB instrument weighs more than 22 kg (50 lb). Two people may be required to lift the instrument out of the shipping carton.
4. Remove the outer plastic wrapping from the system.
5. Manually open the lower door of the system. IMPORTANT: Avoid opening the plate-carrier door more than 90 degrees (horizontal position) as this may permanently damage the door, possibly creating a light leak. Refer to the Instrument Unpacking Instruction Sheet that is included in the instrument carton.
6. There are three foam blocks securing the plate carrier in the system. The foam blocks are positioned as shown in Figure 2.
7. Pull the center foam block from the front of the plate carrier compartment.
8. Reach in the left side of the plate carrier compartment. Grasp the left foam block and remove.
9. Reach in the plate carrier compartment. Grasp the plate carrier and manually move it to the left.
10. Reach in the right side of the plate carrier compartment. Grasp the right foam block and pull it out. DO NOT DISCARD THE FOAM BLOCKS.
11. Proceed to unpack the instrument according to the Instrument Unpacking Instruction Sheet.
12. Consult the manufacturer's Operators Manual for additional details and illustrations.

Figure 2. Placement of Foam Blocks around Plate Carrier (top view)



Installing the Revelation™ Software

Do not yet attach the USB cable to the instrument! The USB drivers will be installed during installation of the Revelation software.

1. Turn on the computer (PC).
2. Start Microsoft Windows.
3. Insert the installation CD. The InstallShield Wizard for Revelation MLX software will start automatically.
4. Click **Next** and follow the Wizard instructions. When the installation is complete, click Finish and remove the installation CD from the PC.
5. Access the Revelation software from the Windows Program File: **Dynex Technologies > Revelation MLX > Revelation MLX.**

Dynex Revelation Software. The end user understands and agrees that the Dynex Revelation™ Software is not a 3M product and is being provided to the end user for the end user's convenience, without charge, on an "AS IS" basis. 3M DISCLAIMS ALL WARRANTIES, EXPRESS OR IMPLIED, INCLUDING THE WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A SPECIFIC PURPOSE, RELATING TO THE DYNEX REVELATION™ SOFTWARE. 3M SHALL NOT BE LIABLE TO END USER UNDER ANY CIRCUMSTANCE FOR ANY DAMAGE OR LOSS OF ANY KIND, INCLUDING DIRECT, INDIRECT, INCIDENTAL, SPECIAL, OR CONSEQUENTIAL DAMAGES, BASED UPON BREACH OF WARRANTY, BREACH OF CONTRACT, NEGLIGENCE, STRICT LIABILITY IN TORT OR ANY OTHER LEGAL THEORY EVEN IF 3M HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING, BUT NOT LIMITED TO, LOSS OF PROFITS, REVENUE, EQUIPMENT, USE, DATA, OR INFORMATION OF ANY KIND.

Connecting the Computer

1. Locate the USB cable.
2. Plug one end of the cable into the USB port at the rear of the MLS instrument.
3. Plug the other end of the cable into a USB port on the computer.

Connecting the Power Cable

Caution! The MLS-USB instrument must be connected to a properly grounded electrical outlet. Obtain assistance from a qualified electrician to verify that your electrical outlets are properly grounded.

1. Locate the power cable for the MLS-USB instrument.
2. Connect the power cable at the rear of the instrument.
3. Connect the MLS-USB instrument to a grounded electrical outlet.

4. PREPARING THE INSTRUMENT FOR INITIAL USE

NOTE: It is important that laboratory safety guidelines are followed while using any chemicals or reagents associated with the MLS-USB instrument.

1. Turn the instrument ON with the power switch on the front lower right-hand corner of the instrument.
2. Turn on the PC.
3. A log-in screen will appear. Press the ENTER key, leaving the Password field blank (the default program setting is not password protected; this may be changed by the user).
4. The instrument will automatically perform a Self-Test of the critical instrument components (X-Y axis, photomultiplier tube [PMT], etc).
5. After the Self-Test is complete, carefully place 3 vials of ATP-Free Water onto the bottle rack (upper right side of the instrument).
6. Insert the Drain Plate (#3912) into the plate carrier such that the green dot is in the upper right corner of the plate carrier (closest to the instrument). Close the plate carrier door.
7. Fill 3 amber vials with ATP-Free Water (from kit # 3005). Load the vials onto the MLS instrument bottle holders.
8. Select **Utilities** from the main menu, then **Dispensers** from the drop-down menu. Click on **Initialize** to prepare the syringes.
9. Once the syringes are initialized, click on **Wash/Purge Injectors.** Select Injectors **A, B, and C** then select **20** strokes. Insert the drain plate into the plate carrier and click **OK** to rinse the injectors.

   NOTE: During the Wash/Purge process, fluid is passed through the reagent tubes and injectors, then into the drain plate. The instrument will not allow the Wash/Purge process to start unless the drain plate is detected on the plate carrier.
10. Watch the movement of fluid within the syringes during the initial operation. It is normal for air to be present in the reagent lines for the first 1 or 2 aspirations, but they should quickly disappear after fluid is drawn into the lines. If bubbles are observed throughout the Wash/Purge process, it may indicate a loose seal in the syringe, or a loose connection between the reagent line(s) and valve, or with the injector probe.
11. When the Wash/Purge process is complete and the plate carrier emerges from the instrument, carefully remove and empty the drain plate.
12. Replace all 3 vials of ATP-Free Water with 3 vials containing 3M MLS Cleaning Solution (from kit #3005).
13. In **Utility/Dispensers**, select **Wash/Purge Injectors.** Select Injectors **A, B, and C** then select **6** strokes. Insert the drain plate in the plate carrier and click **OK**.
14. Carefully remove and empty the drain plate.
15. Replace all 3 vials of Cleaning Solution with 3 vials of ATP-Free Water and **Wash/Purge** injectors **A, B, and C** for **20** strokes.
16. Carefully remove and empty the drain plate.
17. Repeat the Wash/Purge process again (steps 12-16).

The initial instrument set-up is now complete. The instrument is ready to perform the Reagent Control Check assay (refer to section 7).

NOTE: If the instrument will not be used immediately, the 3M MLS Cleaning Solution should remain in the reagent lines. The reagent lines must **always** be rinsed with 20 strokes of ATP-Free Water before the Reagent Control Check or product testing begins.

5. PREPARING THE MLS-USB INSTRUMENT FOR SAMPLE TESTING

This section applies to an MLS-USB instrument that has successfully completed the 'Preparing the Instrument for Initial Use' process (Section 4).

1. Turn on the MLS-USB instrument first, then the PC.
2. A log-in screen will appear. Press the ENTER key, leaving the Password field blank.
3. Wait for the instrument to complete the Self-Test.

5.1 Washing the Injectors

This section assumes that the reagent lines contain 3M MLS Cleaning Solution.

1. After the Self-Test is complete, carefully place 3 vials of ATP-Free Water onto the bottle rack (upper right side of the instrument).
2. Select **Utilities** from the main menu, then **Dispensers** from the drop-down menu. Click on **Initialize** to prepare the syringes.
3. Once the syringes are initialized, click on **Wash/Purge Injectors**. Select Injectors **A, B, and C** then select **20** strokes. Insert the drain plate into the plate carrier and click **OK** to rinse the injectors.

   NOTE: During the Wash/Purge process, fluid is passed through the reagent tubes and injectors, then into the drain plate. The instrument will not allow the Wash/Purge process to start unless the drain plate is detected on the plate carrier.
4. Confirm the movement of fluid within the syringes during the initial operation. It is normal for air to be present in the reagent lines for the first 1 or 2 aspirations, but they should quickly disappear after fluid is drawn into the lines.
5. When the Wash/Purge process is complete and the plate carrier emerges from the instrument, carefully remove and empty the drain plate.
6. Replace all 3 vials of ATP-Free Water with 3 vials containing the 3M MLS Cleaning Solution.
7. In **Utility/Dispensers**, select **Wash/Purge Injectors**. Select Injectors **A, B, and C** then select **6** strokes. Insert the drain plate in the plate carrier and click **OK**.
8. Carefully remove and empty the drain plate.
9. Replace all 3 vials of Cleaning Solution with 3 vials of ATP-Free Water and **Wash/Purge** injectors **A, B, and C** for **20** strokes.
10. Carefully remove and empty the drain plate.

5.2 Priming the Injectors

1. Load the assay reagents (extractant, ATPase, and L/L1) onto the instrument.
2. In the **Utility/Dispensers** menu, select **Prime Injectors**. Select Injectors **A, B, and C** and select **4** strokes.
3. Insert the drain plate in the plate carrier and click **OK**.
4. Once the priming is completed, carefully remove and empty the drain plate.

The instrument is ready to perform the Reagent Control Check (refer to section 7).

6. MLS REAGENT PREPARATION FOR REAGENT CONTROL CHECK AND UHT ASSAY

6.1 General Guidelines for MLS Reagents:

- Rehydrate reagents and allow them to reach ambient temperature prior to use. Cold reagents may produce low RLU readings, and samples may be incorrectly interpreted as sterile (ie, false negative result).
- Do not heat reagents to warm them, as this may inactivate the reagents.
- Store reagents at 2-8ºC when not in use
- Do not freeze reagents
- To avoid ATP contamination, do not touch pipette tips or reagent vial stoppers with bare hands, or any part of the MLS instrument that comes in direct contact with the reagents.

6.2 Preparing the Reagents

L/L1:

1. Open the bottle of lyophilized L/L1 enzyme (green cap). Carefully remove the stopper; the contents are under vacuum.
2. Pipette or pour the entire bottle of L/L1 Buffer (gold cap) into the bottle of L/L1 enzyme.
3. Replace the stopper. Invert the bottle twice, then gently swirl. DO NOT SHAKE!
4. Rehydrated L/L1 reagent is stable for 12 hours at ambient temperature OR for 5 days when stored at 2-8°C.

ATPase:

1. Open the bottle of lyophilized ATPase enzyme (blue cap). Carefully remove the stopper; the contents are under vacuum.
2. Pipette or pour the entire bottle of ATPase Buffer (gold cap) into the bottle of ATPase enzyme.

3. Replace the stopper. Invert the bottle twice, then gently swirl. DO NOT SHAKE!
4. Rehydrated ATPase is stable for 12 hours at ambient temperature OR for 2 days when stored at 2-8°C.

Extractant:

The Extractant solution is ready for immediate use, and is stable at ambient temperature.

Table 1. Summary of MLS Reagent Storage, Use, and Stability

REAGENT	Volume *	# Tests per Bottle *	Storage	Stability		Function
				2-8ºC	Ambient	
Extractant	125 mL	>1200	Ambient		12 months (or expiration on bottle)	Rapidly lyses cell walls and releases microbial ATP
ATPase	Dilute with entire vial of ATPase Buffer (9.5 mL)	150	2-8°C	2 days	12 hrs	Lyses somatic cells, degrades somatic/ free ATP in sample; lowers "background" readings to allow detection of microbial contamination
L/L1	Dilute with entire vial of L/L Buffer (35 mL)	>300	2-8°C	5 days	12 hrs	Reacts with ATP to trigger light reaction
ATP Control	ATP-free H2O (1.0 mL)	20	2-8°C	24 hrs	12 hrs	Tests integrity of instrument and assay reagents

* Volumes and # of tests/bottle are shown for 3M™ MLS UHT Dairy Screen Kits #3000DPQCOG and #3060. Reagent volumes differ for other kits.

7. REAGENT CONTROL CHECK ASSAY

Verify reagent and system performance using an ATP Control Kit (#3004 in US; #LWATP10 or #ATP10 outside of US). A reagent control check should be performed each day, before starting any product testing.

7.1 Preparing the ATP Control

NOTE: This section is written for ATP control #3004. Please refer to the kit insert for ATP standard #LWATP10 and #ATP10.

1. Carefully open a vial of lyophilized ATP; be careful not to touch the rubber stopper. Pipette 1.0 mL of ATP-Free Water into the vial. Replace the stopper and invert the vial 5-10 times. Allow at least 5 minutes for the ATP to rehydrate before use.
   - 1.0 mL of ATP contains sufficient volume for 20 wells, or 5 complete reagent checks
   - Rehydrated ATP solution is stable for 24 hours when stored at 2-8°C.
2. Prepare fresh ATPase and L/L1 OR verify that previously rehydrated reagents were recently prepared. Refer to Section 6 for reagent preparation instructions and stability information.

7.2 Instrument Preparation

This section assumes that the actions described in Section 5 have been completed.

1. Load the assay reagents (extractant, ATPase, and L/L) onto the instrument.
2. In **Utilities/Dispensers**, select **Prime Injectors**. Select injectors **A, B, and C** and **4** strokes of reagent. Click **OK** to prime the reagent lines.
3. Confirm that a drain plate is present in the plate carrier.
4. Carefully remove and empty the drain plate.
5. The instrument is ready to begin the reagent control check.

7.3 Instructions for Reagent Control Check

This section assumes that reagent lines have been primed.

1. Using a strip of 6 microwells (#3008), pipette 50 µL of rehydrated ATP into each of the last 4 microwells (wells C1-F1, leaving wells A1 and B1 empty). Do not deposit ATP onto the sides of the wells.
2. Place the microwell strip into a microwell strip holder (#3009) and place the holder onto the instrument plate carrier, such that Well A1 is in the left upper corner of the plate carrier (closest to the instrument).
3. Click the **Reagent** button on the main menu. Complete the Lot Information section if desired.
4. Enter the file name and user information, then click **OK** to start the reagent control check. The assay is pre-programmed for 6 test wells and ATP control in wells C1-F1.
5. Results will be available within approximately 3 minutes.
6. Following favorable reagent check results, the instrument and reagents are ready for use.
7. Perform UHT Dairy Product Sterility Testing according to Section 8 or your user-specific protocol.

7.4 Interpretation of Results

In general, successful reagent control check results are as follows:

Wells A1 and B1 contain ATPase, Extractant and L/L1, and serve as a negative control (no ATP).

- Readings of **<35 RLU** in both wells indicate that there is no contamination in the instrument or reagents.

Wells C1 and D1 contain ATP, ATPase, Extractant, and L/L1.

- Readings of **<150 RLU** in both wells indicate that the ATPase enzyme is active.

Wells E1 and F1 contain ATP, Extractant, and L/L1 (no ATPase), and serves as a positive control.

- Readings of **>4000 RLU** in both wells indicate that the L/L1 reagent and ATP control are active.

NOTE: The Pass criteria shown above pertain to the ATP Reagent Control Kit #3004. Pass criteria for ATP System Control Reagents #LWATP10 and #ATP10 are shown in Table 2 below. Pass/Fail criteria for reagent control checks and UHT testing may vary depending on product or user requirements.

Table 2. Default "Pass" Values for the Reagent Control Check

WELL	For 3M MLS ATP Reagent Control Kit #3004 **RLU**	For 3M MLS ATP System Control Reagent #LWATP10 or ATP10 **RLU**
A1	< 35	< 35
B1	< 35	< 35
C1	< 150	< 35
D1	< 150	< 35
E1	> 4000	> 1500
F1	> 4000	> 1500

7.5 Troubleshooting Tips

1. If the reagent check produces an unsatisfactory reading, repeat the procedure with a fresh bottle of ATP and/or ATPase and L/L1. Ensure that the reagent lines are primed. If the reagent check fails again, it may indicate a problem with one or more reagents, or with the MLS instrument itself.
2. Check the expiration date on all reagent bottles, and ensure that the reagents have been properly stored. The shelf-life of rehydrated reagents are as follows:
   - ATPase = 2 days at 2-8°C
   - L/L1 = 5 days at 2-8°C
   - ATP control = 24 hours at 2-8°C

Refer to Appendix II for additional troubleshooting tips.

8. UHT DAIRY PRODUCT STERILITY TESTING

3M MLS UHT Dairy Screen Kits are available for use with this instrument. These kits utilize ATP bioluminescence to detect microbial (contaminant) ATP in sterile dairy and dairy-type products. The kit reagents are designed to exclude ATP from non-microbial sources and measure only microbial ATP. Detection of microbial ATP (outside appropriate Pass/Fail criteria) indicates microbial contamination in the product.

8.1 Preparation of Test Sample(s)

1. Incubate the test product in its original unopened container for the appropriate time and temperature specified in the user's testing protocol.
2. After incubation, shake the test product to homogenize the sample. Carefully open the package(s).

8.2 Assay Set-Up

1. Prepare reagents (Section 6)
2. Perform a Reagent Control Check (Section 7) before product testing.
3. Pipette 50 µL of the first sample into well A1 of a Microwell Plate (#3007). Use a fresh pipette tip for each sample. Do not deposit the sample onto the sides of the wells.
4. Repeat Step 3 for all samples. Always dispense samples in the correct order (wells A1 to H1, then A2 to H2, and so on).
5. Load the reagents onto the instrument as follows:
   - Extractant at injector position **A**
   - ATPase at injector position **B**
   - L/L1 at injector position **C**
6. Place the assay plate with samples loaded into the instrument, such that well A1 is in the upper left corner of the plate carrier (closest to the instrument).
7. Select **UHT Assay** on the menu bar and enter the file name, operator, and number of samples to be tested. Click **Start** to begin the assay.
8. Assay results will be displayed in Relative Light Units (RLU) after all samples are analyzed (40 minutes for 96 samples).
9. If desired, a negative control sample (example: sterile sample or non-incubated sample) may be included with the test samples. A positive control sample (example: raw milk) may also be included.
10. When the day's testing is complete, rinse the reagent lines with ATP-free water and Cleaning Solution according to the daily cleaning procedure (see Section 9).

8.3 Interpretation of Results:

As a guideline, a reading of <150 RLU is a Pass result; however, Pass/Fail criteria may vary by product and customer requirements. Contact your Technical Service Representative for assistance.

9. CLEANING THE INSTRUMENT AFTER TESTING

With normal use, microbial contamination and biofilm formation can occur in the MLS instrument. The 3M MLS Injector Cleaning Kit (#3005) helps to prevent microbial contamination and biofilm formation in the injectors and reagent lines, thus avoiding high background readings, false positive results, and inaccurate dispensing of reagents. To properly maintain your MLS instrument, use the 3M MLS Injector Cleaning Kit at least once every day.

1. Load 3 vials of ATP-Free Water onto the MLS instrument bottle holders.
2. In the **Utility/Dispensers** menu, select **Wash/Purge Injector**s. Select Injectors **A, B, and C** and select **20** strokes. Insert the drain plate in the plate carrier and click **OK** to rinse the injector(s).
3. Once the wash cycle is complete, remove and empty the drain plate.
4. Replace all 3 vials of ATP-Free Water with 3 vials containing the 3M MLS Cleaning Solution.
5. In the **Utility/Dispensers** menu, select **Wash/Purge Injectors**. Select Injectors **A, B, and C** and select **6** strokes. Insert the drain plate in the plate carrier and click **OK** to wash the injectors.

6. Carefully remove and empty the drain plate.
7. Replace all 3 vials of Cleaning Solution with 3 vials of ATP-Free Water and wash/purge reagent lines **A, B, and C** for **20** strokes. Carefully remove and empty the drain plate.

NOTE: When the instrument is not in use, the 3M MLS Cleaning Solution should remain in the reagent lines. **Always rinse the reagent lines with 20 strokes of ATP-Free Water before the Reagent Control Check or product testing begins.**

10. SHUTTING THE SYSTEM DOWN

1. To close the Revelation software, click on the **X** at the upper right corner of the software screen. A message will appear asking if you want to save results. Select **Yes** for the desired sets of results.
2. In the **Start** menu on the lower left corner of the computer screen, select Shut Down. Wait until the message appears
3. After the OK to Shut Down message appears, turn the MLS-USB instrument OFF using with the power switch on the front lower right-hand corner of the instrument.

11. MAINTENANCE CLEANING

With normal use, microbial contamination and biofilm formation can occur in the MLS instrument. The 3M MLS Maintenance Solution (#3006 in US; #BMLSCK outside US) removes protein residues and microbial contamination in the injectors and reagent lines, thus avoiding high background readings, false positive results, and inaccurate dispensation of reagents. To properly maintain your MLS instrument, use the 3M MLS Maintenance protein removal solution at least once every week.

Important Safety Note: Caution - This 3M MLS Maintenance Solution is CORROSIVE. Please read the Material Safety Data Sheet before using this kit for the first time. Take all necessary precautions. Wear eye protection, gloves, and protective clothing. Take care when emptying the drain plate.

1. Load 3 vials of ATP-Free Water (from the 3M MLS Injector Cleaning Kit #3005) onto the instrument bottle holders.
2. In the **Utility/Dispensers** menu, select **Wash/Purge Injectors**. Select Injectors **A, B, and C** and select **20** strokes. Insert the drain plate in the plate carrier and click **OK** to rinse the injector(s).
3. Once the wash cycle is complete, remove and empty the drain plate.
4. Replace the vials of ATP-Free Water with 3 vials containing the 3M MLS Maintenance Kit protein removal solution.
5. In the **Utility/Dispensers** menu, select **Wash/Purge Injectors**. Select Injectors **A, B, and C** and select **6** strokes. Insert the drain plate in the plate carrier and click **OK** to wash the injectors.
6. Carefully remove and empty the drain plate. Allow the protein removal solution to remain in the reagent lines for at least 20 minutes.
7. Replace the vials of protein removal solution with 3 vials of ATP-Free Water and wash/purge reagent lines **A, B, and C** for **20** strokes. Carefully remove and empty the drain plate.
   NOTE: A red-colored precipitate may temporarily appear when the Maintenance Solution comes into contact with the Cleaning Solution or BDK extractant. If this is observed, it is essential to rinse the reagent lines thoroughly using ATP-Free Water before priming the instrument with reagents. Refer to Section 9 for cleaning instructions.
8. Perform a Reagent Control Check before testing samples (Section 7).

Table 3 summarizes the recommended routine maintenance of the MLS-USB instrument.

Table 3. Routine Maintenance Schedule for MLS-USB Users

RECOMMENDED ACTION	FREQUENCY	
	DAILY	WEEKLY
Rinse the reagent lines with ATP-free water (upon start-up and between UHT Assays	X	
Clean the lines using Injector Cleaning Solution and ATP-free water	X	
Clean Injectors A, B, and C with Maintenance Solution		X

12. MOVING THE MLS-USB INSTRUMENT TO ANOTHER WORKBENCH

1. Remove the reagents from the bottle holders.
2. Verify that reagent tubing is securely in place.
3. Open the door to the plate carrier and place the 3 foam shipping blocks around the plate carrier (refer to the Instrument Unpacking Instruction sheet).
4. While moving the instrument, keep it in an upright and level position.
5. Carefully place the instrument onto the desired surface. Open the door to the plate carrier and remove the foam shipping blocks around the plate carrier.
6. Clean and maintain the reagent lines in 3M MLS Cleaning Solution until the next testing (Section 9).

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APPENDIX I
REAGENT USAGE CALCULATIONS *

Extractant

Bottle contains 125 mL

1.5 mL needed for system priming + 0.5 mL for back-up in the line

125 mL - 2.0 mL for priming/back-up = 123 mL of usable Extractant

Each UHT test requires ~100 µL, therefore 1 mL = 10 tests

123 mL Extractant x 10 tests / mL = >1200 tests per bottle

ATPase

Rehydrated bottle contains 9.5 mL

1.5 mL needed for system priming + 0.5 mL for back-up in the line

9.5mL - 2.0 mL for priming/back-up = 7.5 mL of usable ATPase

Each UHT test requires 50 µL, therefore 1 mL = 20 tests

7.5 mL ATPase x 20 tests / mL = 150 tests per bottle

L/L1

Rehydrated bottle contains 35 mL

1.5 mL needed for system priming + 1.5 mL for back-up in the line

35 mL - 3.0 mL for priming/back-up = 32 mL of usable L/L1

Each UHT test requires ~100 µL, therefore 1 mL = 10 tests

32 mL L/L x 10 tests / mL = 320 tests per bottle

Graduations are shown on the ATPase and L/L1 reagent bottles, allowing the user to view the approximate number of tests remaining in each reagent bottle.

* Calculations pertain to 3M MLS UHT Dairy Screen Kits #3000DPQCOG and #3060. Reagent volumes differ for other kits.

APPENDIX II
REAGENT CONTROL CHECK – TROUBLESHOOTING OVERVIEW

CONTROL WELLS	WELL CONTENTS	TESTS FOR:	PASS CRITERIA * (RLU)	RESULTS SCENARIOS: A	B	C	D	E	F	G
A1	Ext, ATPase, L/L1 (no ATP)	Reagent contamination	<35	6	**36**	5	7	9	**154**	4
B1			<35	5	**37**	7	8	7	**189**	8
C1	Ext, ATPase, L/L1, ATP	ATPase activity	<150	8	**155**	6	**158**	**4100**	**204**	11
D1			<150	9	**165**	9	**97**	**2950**	**132**	12
E1	Ext, L/L1, ATP (no ATPase)	ATP control, L/L1 activity	>4000	5653	5127	**6**	5276	5348	**10578**	**3800**
F1			>4000	5240	5497	**9**	4930	5067	**14573**	**3100**
				Results OK	**Potential Problem:**	**Potential Problem:**	**Potential Problem:**	**Potential Problem:**	**Potential Problem:**	**Potential Problem:**
					* Wells A1-D1 are marginal failures, may indicate a problem with contamination or biofilm in reagent lines	* L/L1 not properly dispensed * No ATP control in wells E1, F1 * Well strip placed horizontally in holder	* ATP control left on the wall of wells C1, D1 * Improper pipetting technique	* ATPase not properly dispensed * ATPase inactive	* Contamination or biofilm in reagent lines	* Decreased ATP control and/or L/L1 activity due to high ambient temperature * C-probe not dispensing properly
					Potential Solutions:	**Potential Solutions:**	**Potential Solutions:**	**Potential Solutions:**	**Potential Solutions:**	**Potential Solutions:**
					* Remove reagents <u>without</u> recovering * Replace amber bottles and solutions; rinse reagent lines with fresh ATP-Free Water and Cleaning Solution * Maintenance Procedure * Repeat reagent control check	* Hydraulic System Check on C-probe: verify proper dispensing * Repeat reagent control check	* Repeat Reagent Check, take care to pipette ATP control into **bottom** of microwells * Repeat reagent control check	* Hydraulic System Check on B-probe: verify proper dispensing * Replace ATPase * Repeat reagent control check	* Remove reagents <u>without</u> recovering * Replace amber bottles and solutions; rinse reagent lines with fresh ATP-Free Water and Cleaning Solution * Maintenance Procedure * Repeat reagent control check	* Check temperature of lab and MLS; should be <25°C * Repeat reagent control check using new ATP control and/or L/L1 * Maintenance Procedure

* Pass criteria pertain to the ATP Reagent Control Kit #3004. Please refer to the kit insert for ATP standard #LWATP10 and #ATP10.