



Product Instructions

DPQCOG3000/3060

 **(EN)** Microbial Luminescence System Ultra High Temperature Dairy Screen Kit

Product Instructions

Microbial Luminescence System Ultra High Temperature Dairy Screen Kit

Product Description and Intended Use

The Neogen® Microbial Luminescence System (MLS) Ultra High Temperature (UHT) Dairy Screen Kit is intended for use with the Neogen® Microbial Luminescence System (MLS) II Instrument. This kit offers a rapid detection system that utilizes the adenosine triphosphate (ATP) bioluminescence technology to detect the presence of microbial ATP in dairy and dairy substitute products that have been processed for commercial sterilization including Ultra High Temperature (UHT) and Extended Shelf Life (ESL) products. After the product is subjected to an enrichment step in its original unopened container, the Neogen MLS UHT Dairy Screen Kit has the capability to exclude ATP from non-microbial sources and then measure only the ATP released by microorganisms. In less than 30 minutes, the microbial ATP is measured in a light output format of Relative Light Units (RLU) proportional to the amount of microbial ATP present in the enriched sample. The Neogen MLS UHT Dairy Screen Kit significantly reduces the time for product release compared to traditional microbiology testing methods.

Neogen Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

Safety

The user should read, understand, and follow all safety information in the instructions for the Neogen MLS UHT Dairy Screen Kit. Retain the safety instructions for future reference.

NOTICE: Indicates a potentially hazardous situation which, if not avoided, could result in property damage.

NOTICE

To reduce the risk of inaccurate results:

- The dehydrated ATPase and LL1 reagents and corresponding buffers should be stored at 2-8°C.
- Avoid the exposure of the kit's reagents to the direct sunlight.
- Do not use reconstituted ATPase and reconstituted LL1 beyond 7 days, stored at 2-8°C when not in use.
- Do not expose reconstituted reagents to ambient temperatures (20-25°C) for more than 12 hours during the 7 days reconstituted ATPase and LL1 shelf life.
- Do not shake the reconstituted reagents. Follow instructions for use described for reagent preparation.

To reduce the risk associated with exposure to chemicals and biohazards:

- Handle enriched samples following standard laboratory safety practices, including wearing appropriate protective apparel and eye protection.

To reduce the risks associated with cross-contamination while preparing the assay:

- The use of gloves is recommended.
- ATP is a common substance. It is present on skin, hair and on many surfaces. To avoid ATP contamination, do not touch pipette tips or reagent vial stoppers with bare hands, or any part of the Neogen MLS II Instrument that comes in direct contact with the reagents.

Consult the Safety Data Sheet for additional information.

For information on documentation of product performance, visit our website at www.neogen.com or contact your local Neogen representative or distributor.

User Responsibility

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at www.neogen.com or contact your local Neogen representative or distributor for more information.

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. The food sample itself may influence results.

It is the user's responsibility in selecting any test method or product 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 any Neogen Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

Limitation of Warranties / Limited Remedy

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, NEOGEN DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any Neogen Food Safety Product is defective, Neogen or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify Neogen within sixty days of discovery of any suspected defects in a product and return it to Neogen. Please contact your Neogen representative or authorized Neogen distributor for any further questions.

Limitation of Neogen Liability

NEOGEN WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall Neogen's liability under any legal theory exceed the purchase price of the product alleged to be defective.

Storage and Disposal

See Table 1 for specific storage conditions. DO NOT FREEZE.

Neogen MLS UHT Dairy Screen Kit components should not be used past the expiration date. Expiration date and lot number are noted on the outside label of the box.

Dispose according to current local/regional/national/industry standards and regulations.

Table 1. Kit contents for 3060 (The DPQCOG3000 contains five 3060 Kits).*

Item	Description	Quantity	Storage	Comments
ATPase (orange cap)	Purified lyophilized ATPase enzyme that degrades somatic cells and free ATP to allow detection of only microbial ATP	2 vials with lyophilized enzyme	2-8°C DO NOT FREEZE	Reconstitute with ATPase buffer (read instructions below)
ATPase Buffer (orange cap)	Buffer to reconstitute ATPase enzyme	2 vials	2-8°C DO NOT FREEZE	Used to reconstitute the ATPase lyophilized enzyme (read instructions below)
Extractant (black cap)	Rapidly lyses microorganisms to release microbial ATP	2 vials	20-25°C DO NOT FREEZE	Ready to use NOTE: Refrigerate until first use. Once in use maintain at 20-25°C. Store in the dark.
LL1 (green cap)	Purified Luciferin/Luciferase complex that interacts with microbial ATP to produce light captured by the Neogen MLS II Instrument	2 vials with lyophilized enzyme	2-8°C DO NOT FREEZE	Reconstitute with LL1 buffer (read instructions below)
LL1 Buffer (green cap)	Buffer to reconstitute LL1 enzyme	2 vials	2-8°C DO NOT FREEZE	Used to reconstitute the LL1 lyophilized enzyme (read instructions below)

* NOTE: The 3060 and the DPQCOG3000 can be utilized to perform up to 600 and 3000 tests respectively. Reconstitute one vial of LL1 and ATPase with the respective buffer to run up to 300 tests.

The following table describes accessory products related to Neogen MLS II that are not included with the Neogen MLS UHT Dairy Screen Kit.

**Table 2.** Neogen Microbial Luminescence System II Products.

Catalog Number	Description
ATP10	Neogen® Clean-Trace® Surface Positive Control (ATP10)
3004	Neogen® Microbial Luminescence System (MLS) ATP Reagent Control
3005	Neogen® Microbial Luminescence System (MLS) Injector Cleaning Kit
3006	Neogen® Microbial Luminescence System (MLS) Maintenance Solution
3007	Neogen® Microbial Luminescence System (MLS) Microwell Plate
3008	Neogen® Microbial Luminescence System (MLS) Microwell Strip
3009	Neogen® Microbial Luminescence System (MLS) Microwell Strip Holder
BMLSCK	Neogen® Microbial Luminescence System (MLS) Weekly Cleaning Kit

Instructions for Use

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

The overall work flow of all procedures to run the Neogen MLS UHT Dairy Screen Kit is described in Figure 1 at the end of this document.

Sample Enrichment

Prior to the screening of UHT or ESL dairy products with the Neogen MLS UHT Dairy Screen Kit, samples need to be enriched to allow growth of low populations of microorganisms.

1. Place the beverage in the incubator. To achieve uniform temperature within the beverage container, ensure that containers do not touch each other or the walls of the incubator. **It is the user's responsibility to validate the enrichment protocol to ensure the results meet the user's criteria.**
2. Following enrichment, remove the beverage container from the incubator.
3. Proceed with sample screening with the Neogen MLS UHT Dairy Screen Kit.

Reagent Preparation

ATPase Reagent**

1. Open the ATPase vial (orange cap) containing the lyophilized ATPase enzyme. Carefully remove the rubber stopper; contents are under vacuum.
2. Open the ATPase buffer vial (orange cap) and pour or pipette the entire contents of the buffer into the ATPase vial.
3. Replace the rubber stopper and invert 5 - 10 times to dissolve the lyophilized ATPase, then gently swirl. DO NOT SHAKE.

****NOTE:** The reconstituted ATPase has a shelf life of 7 days when stored at 2-8°C including up to 12 hours at 20-25°C. Return the reconstituted ATPase to storage at 2-8°C when not in use. **Do not freeze the reconstituted ATPase.**

LL1 Reagent**

1. Open the LL1 vial (green cap) containing the lyophilized Luciferin/Luciferase complex. Carefully remove the rubber stopper; contents are under vacuum.
2. Open the LL1 buffer vial (green cap) and pour or pipette the entire contents of the buffer into the LL1 vial.
3. Replace the rubber stopper and invert 5 times to dissolve the lyophilized LL1, then gently swirl. DO NOT SHAKE.

****NOTE:** The reconstituted LL1 has a shelf life of 7 days when stored at 2-8°C including up to 12 h at 20-25°C. Return the reconstituted ATPase and LL1 Reagents to storage at 2-8°C when not in use. **Do not freeze the reconstituted LL1.**

Extractant (Black cap)

The Extractant solution is ready for immediate use. Once in use, it can be maintained at 20-25°C. It is not recommended to return to the refrigerator. When not in use store in a dark place.



Preparing the Neogen MLS II Instrument

a. Cleaning the system for initial use

1. Switch on the Neogen MLS II Instrument first and then the computer.
2. Launch the Neogen® Microbial Luminescence System (MLS) II Software.
3. Place three vials containing Neogen MLS Cleaning Solution on the Neogen MLS II Instrument injectors.
4. In the Neogen MLS II Software, open the **“Actual”** tab and run a **“Wash Assay”**.
5. Replace the three vials containing Neogen MLS Cleaning Solution with three vials containing ATP-free water and run a second **“Wash Assay”**.

Refer to the Neogen MLS II User's Manual for detailed information related to cleaning the Neogen MLS II Instrument injectors.

b. Priming the Reagents in the Neogen MLS II Instrument Injector Lines

Important: Ensure all reagents (reconstituted ATPase, Extractant and reconstituted LL1) have reached ambient temperature (20-25°C) before starting the Reagent Control Assay and UHT Assay.

1. Replace vials containing ATP-free water with reconstituted ATPase (on injector A), Extractant (on injector B) and reconstituted LL1 (on injector C). Retain rubber stoppers and caps for resealing vials for later storage.
2. In the Neogen MLS II Software, open the **“Actual”** tab and run a **“Prime Assay”**.

For more detailed information related to priming of the Neogen MLS II Instrument refer to the Neogen MLS II User's Manual.

NOTE: Priming injectors before running a Reagent Control Assay and Dairy Screen Assay is needed to ensure the instrument's injectors and reagent tubes are pre-filled with the Neogen MLS UHT Dairy Screen Kit Reagents. Failure to do so can result in False-Negatives or False-Positive results.

c. Running a Reagent Control Assay

Performing a Reagent Control Assay is always recommended prior to product testing, to ensure the Neogen MLS II Instrument and Reagents are performing appropriately. Please refer to the Neogen MLS ATP Reagent Control or Neogen Clean-Trace Surface Positive Control (ATP10) Instructions for Use for detailed information about the Reagent Control Assay and reconstitution of the ATP Reagent.

1. Place a Neogen MLS Microwell Strip (a minimum of 6 wells is required) in a Neogen Microwell Strip Holder. A Neogen MLS Microwell Plate can be used instead of the Neogen MLS Microwell Strips.
2. Pipette 50 µL of the reconstituted ATP Reagent (ATP10 or 3004 see Table 2) into the **bottom** of the last 4 wells (C1, D1, E1, and F1). Wells A1 and B1 should be empty.
3. In the Neogen MLS II Software, open the **“Actual”** tab and run a **“Reagent Control Assay”**.

For more detailed information about the Reagent Control Assay and interpretation of results, please refer to the Neogen MLS ATP Reagent Control or Neogen Clean-Trace Surface Positive Control (ATP10) Instructions for Use.

Running a UHT Assay

1. Homogenize the enriched beverage in its original unopened container by shaking.
2. Aseptically, remove 50 µL of the enriched beverage with a micropipette and deposit the sample into the **bottom** of a well in a Neogen MLS Microwell Plate (a Neogen MLS Microwell Strip can be utilized for fewer number of assays).

NOTE. Careful pipetting of samples is required to ensure accurate results. Depositing the sample onto the well wall can cause results to be inaccurate. Users should pipette samples directly into the **bottom** of the well.

3. Repeat this step utilizing a fresh pipette tip for each sample. It is recommended to dispense the sample in order of columns A1 through H1, A2 through H2 etc.
4. In the Neogen MLS II Software, open the **“Actual”** tab and select the **UHT Assay.V1**
5. If desired, complete the Neogen MLS UHT Dairy Screen Kit Lot information and click **“OK”**
6. Select the wells that contain samples to be analyzed in the 96 well grid displayed on the **“Actual”** tab (unselected wells will not be analyzed).
7. Click **“Start”**
8. Complete the **“Load Plate”** information (name file of the Assay).
9. Carefully place the Neogen MLS Microwell Plate containing the samples onto the plate carrier of the Neogen MLS II Instrument ensuring well 'A1' is positioned at the left corner of the plate carrier, closest to the user.



10. Click “OK” and the assay will start.

11. After the Assay is completed, remove the Neogen MLS Microwell Plate from the Neogen MLS II Instrument and dispose the plate according to current industry standards.

Interpretation of Results

NOTE. It is the user’s responsibility to validate the Pass/Fail limits to ensure this test method meets the user’s criteria. To establish the threshold/background RLU, it is advised to obtain repeated measurements (multiple replicates) of RLU readings from several lots of non-contaminated product. Utilize this data to determine the standard deviation of the background RLU of the analyzed product. *A minimum Pass/Fail limit can be defined as the average background RLU plus three times the standard deviation of the background RLU for each specific matrix/product.* For specific instructions on changing the Pass/Fail limits in the software, contact your Neogen Technical Service representative.

1. Results will appear after approximately 27 minutes (for a full plate of 96 samples) in the “Report” Tab. The results will be displayed by means of color code as the assay progresses (Green/Pass and Red/Fail). Placing the mouse over the well will show the RLU value in real time as the assay progresses.
2. A Pass or Fail result is determined by the establishment of a Pass/Fail limit based on a RLU threshold value. As a default in the software, a result of <150 RLU will be marked as a Pass; however, Pass/Fail limits may vary depending on customer or product requirements.

Cleaning the Neogen MLS II Instrument after use

Following sample testing, the reagents dispensing lines and injectors must be rinsed by flushing ATP-free water through the system.

1. Replace the ATPase vial, Extractant and LL1 vial with vials of ATP-free water. Place rubber stopper/caps back onto their respective vials and store the ATPase and LL1 Reagents at 2-8°C or discard appropriately. Store Extractant at 20-25°C after first use (Table 1).
2. Select the “Actual” tab and click on the “Wash Assay” with ATP-free water first and then repeat with Neogen MLS Cleaning solution. Leave the Neogen MLS Cleaning solution on the instrument until next use.

Refer to the Neogen MLS User’s Manual for detailed information related to cleaning the Neogen MLS II Instrument injectors.

NOTE. The waste container for the Neogen MLS II Instrument should be emptied on a daily basis.

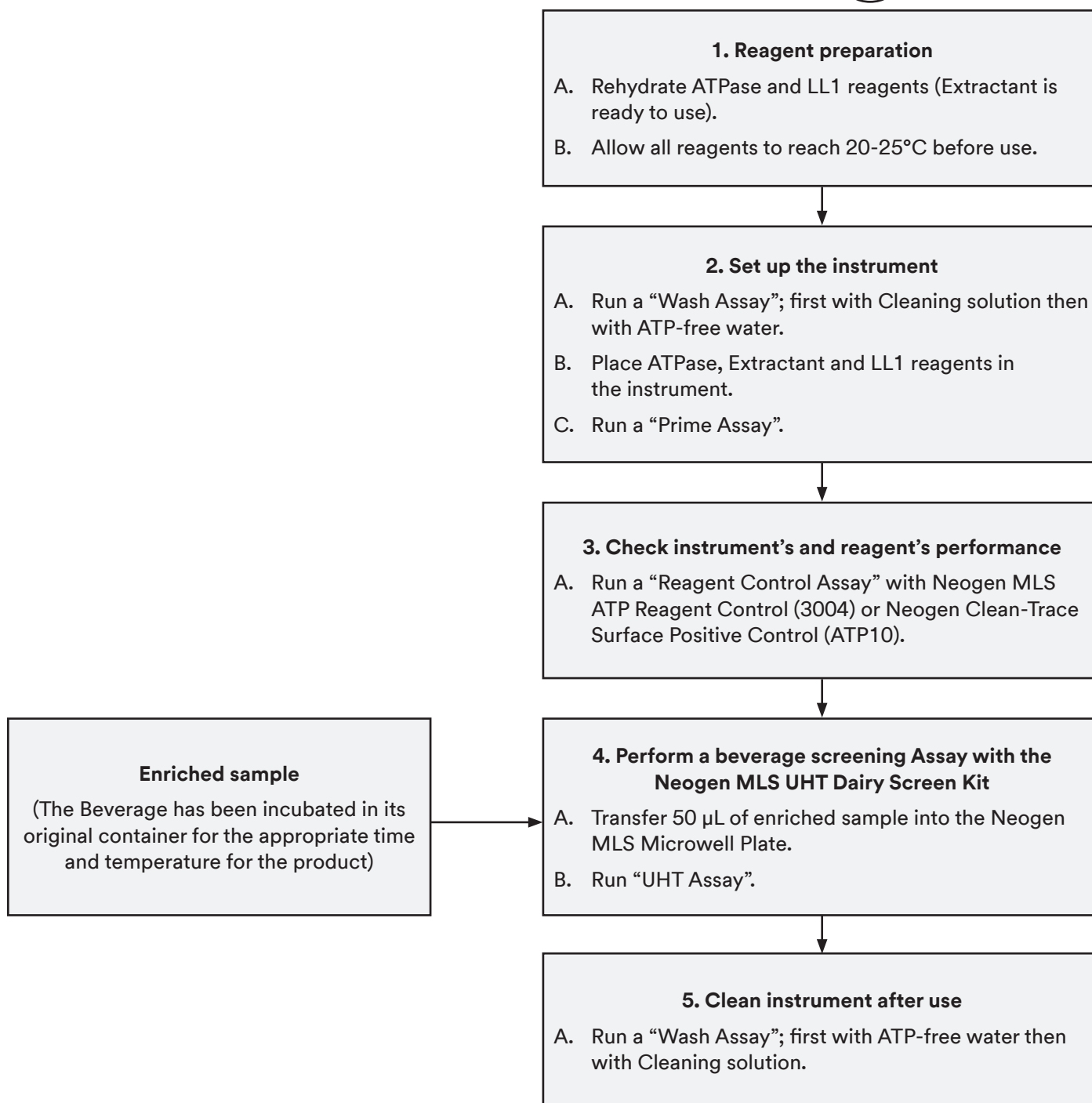


Figure 1. Work flow to perform screening of UHT and ESL dairy or dairy substitute products.

If you have questions about specific applications or procedures, please visit our website at www.neogen.com or contact your local Neogen representative or distributor.

Explanation of Symbols

info.neogen.com/symbols

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