

# NOVEO

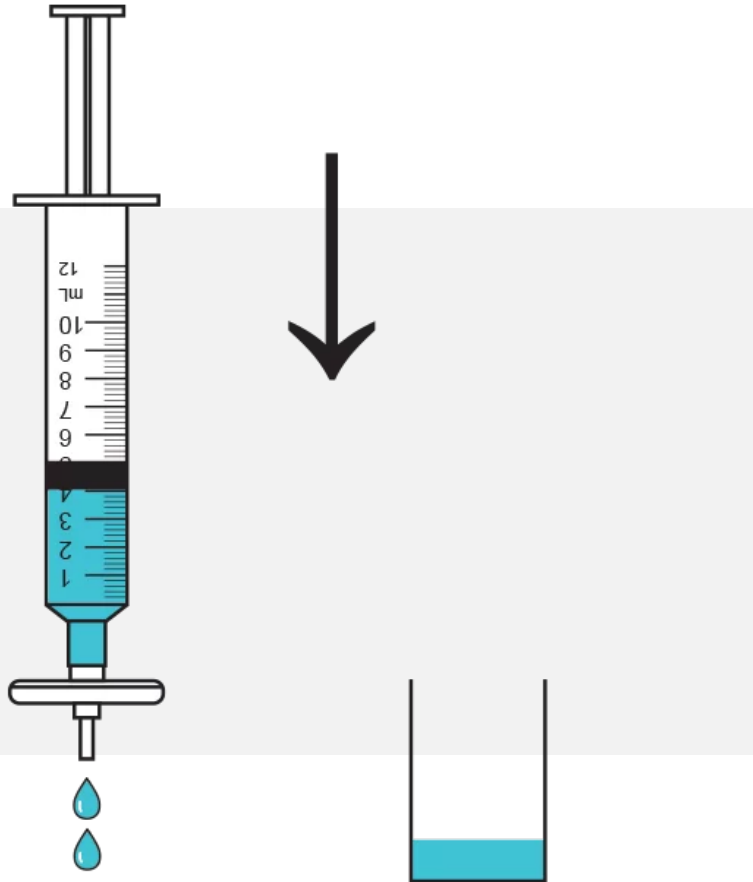
## 2 Min Water Control Monitoring

Instructions for Use –  
Kikkoman Lumitester Smart  
Noveo 2-Min Control Kit

**kikkoman**  
Kikkoman Biochemifa Company

GLBIOCONTROL  
RAPID MICROBIAL DIAGNOSTIC





## Lumitester & Water Control Kits

- ✓ Lumitester Smart
  1. What's in the box
  2. Instruction manual
  3. Cleaning & maintenance
  
- ✓ Water Control Monitoring
  1. What's in the boxes
  2. Setup for water testing
  3. Processing a sample
  4. Troubleshooting



# Lumitester Smart

What's inside the box

## Package contents



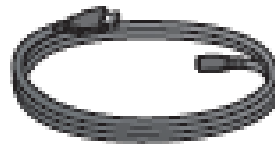
1 Lumitester Smart



1 Strap



2 size AA alkaline  
batteries  
(for operation check)



1 USB cable



3 cleaning brushes



1 Quick manual

# Lumitester Smart

## Instructions Manual

Electronic copies of the Lumitester Smart Quick Manual and Instruction Manual can be downloaded from:  
<https://biochemifa.kikkoman.com/e/support/dl/smart/>

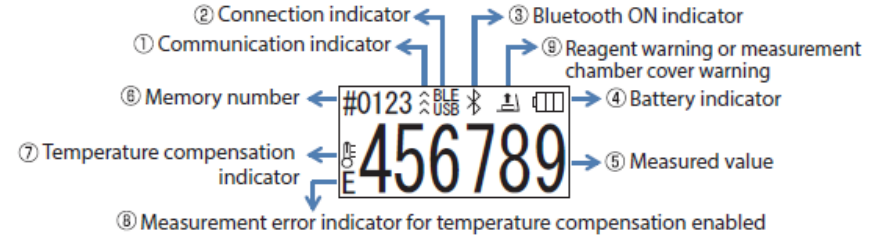
# 1 Names and Functions

## 1-1. Product



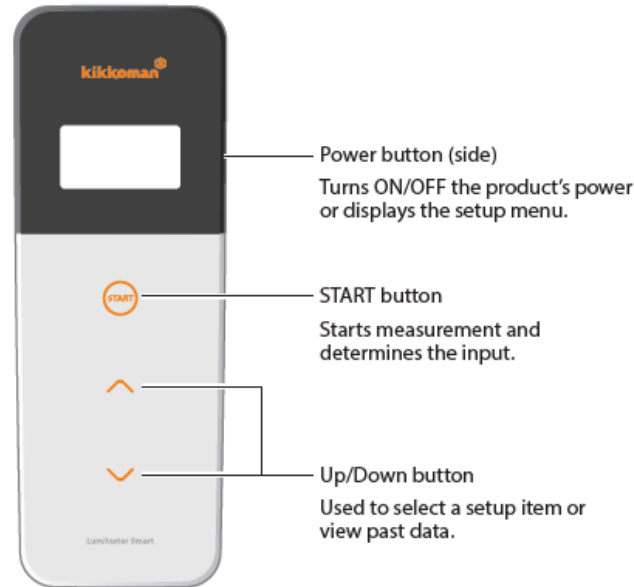
① Display Unit	Panel that displays the results of measurement, the mode number, etc. Refer to "1-2. Display Unit" (→P4).
② Operation Unit	Panel for measurements or setting operation. Refer to "1-3. Operation button" (→P5).
③ Power Button	Turns ON/OFF the power. Displays the setup menu.
④ Stand	Used to set the product in an upright position.
⑤ Measurement chamber	Chamber where reagent is inserted.
⑥ USB port	Cover for the USB connector. Connector that is used for PC connection.
⑦ Measurement chamber cover	Cover for measurement chamber.
⑧ Battery cover	Cover used to insert and remove batteries and switch Bluetooth ON/OFF.

## 1-2. Display Unit



① Communication indicator	Indicates communication with smartphone, tablet, and/or PC.										
② Connection indicator	Indicates connection with smartphone, tablet, and/or PC. <table border="1"> <thead> <tr> <th>Target</th><th>Display</th></tr> </thead> <tbody> <tr> <td>Bluetooth</td><td></td></tr> <tr> <td>USB</td><td></td></tr> </tbody> </table>	Target	Display	Bluetooth		USB					
Target	Display										
Bluetooth											
USB											
③ Bluetooth ON indicator	Indicates that the Bluetooth ON/OFF switch of the product is ON.										
④ Battery indicator	Displays the remaining battery level. <table border="1"> <thead> <tr> <th>Battery indicator</th><th>Display</th></tr> </thead> <tbody> <tr> <td>Full</td><td></td></tr> <tr> <td>High</td><td></td></tr> <tr> <td>Medium</td><td></td></tr> <tr> <td>Low battery</td><td></td></tr> </tbody> </table>	Battery indicator	Display	Full		High		Medium		Low battery	
Battery indicator	Display										
Full											
High											
Medium											
Low battery											
⑤ Measured value	The measured value is displayed up to 6 digits with right justification.										
⑥ Memory number	Shows the measured data number with # + 4 digits.										
⑦ Temperature compensation indicator	Indicates that the temperature compensation setting is ON.										
⑧ Measurement error indicator for temperature compensation enabled	If the temperature compensation is ON, the error code "E047" or "E048" is displayed at the temperature 40°C or higher or 10°C or lower, respectively, during measurement countdown, and the measurement result without temperature compensation is displayed with the error "E".										
⑨ Reagent warning or measurement chamber cover warning	Indicates that you must close the measurement chamber cover, or remove or insert the reagent. <table border="1"> <thead> <tr> <th>Display</th><th>Instruction</th></tr> </thead> <tbody> <tr> <td></td><td>Insert the reagent or close the measurement chamber cover.</td></tr> <tr> <td></td><td>Remove the reagent.</td></tr> </tbody> </table>	Display	Instruction		Insert the reagent or close the measurement chamber cover.		Remove the reagent.				
Display	Instruction										
	Insert the reagent or close the measurement chamber cover.										
	Remove the reagent.										

## 1-3. Operation button



To adjust the sensitivity of the START and/or up/down buttons, refer to "6-4. Switch sensitivity" (→P30).

### Caution

- As the START and up/down buttons adopt electrostatic capacity switches, they may not react when operated by a wet hand or through a thick glove.

## 2









## For safe and proper use






- This product is designed for ATP Swab Test. Do not use this product for any other use. Please read carefully for proper usage of the product.

Please read this section carefully beforehand to use the product correctly. The warning messages listed here indicate important safety issues as well as failures and malfunctions.

**Caution** Failure to properly handle the product may lead to injuries or damages.

**Prohibited** Indicates what must NOT be done to use the product.

Caution	
 <p>Do not disassemble or remodel the product. Fire, electric shock, or failure may occur.</p>	 <p>Do not expose the product to water or operate it with wet hands. Failure to do so may cause electric shock or malfunction.</p>
 <p>Do not push nor rub the display and operation panel with hard or sharp objects. Injury or failure may occur.</p>	 <p>Do not use batteries other than the specified ones (size AA alkaline batteries or charged size AA nickel-hydride batteries). Fire, injury, or failure may occur.</p>
 <p>Do not apply organic solvent to clean the product. Fire, electric shock, or failure may occur.</p>	 <p>Remove the batteries when the product is to be stored for an extended period of time. Failure to do so may cause liquid leak and burst.</p>
 <p>Do not use nor store the product exposed to direct sunlight, dust, and/or high temperature/humidity. Fire or failure may occur.</p>	 <p>Do not place the product where it gets hot (near the fire, near the heater, or under the scorching sun). Fire, burn, or failure may occur.</p>

For proper use	
 <p>Do not apply too much force to the stand and/or measurement chamber cover, or move the product while they are open. The stand and/or the measurement chamber cover may be damaged.</p>	 <p>Do not drop nor apply excess impact. Failure to do so may cause malfunction or poor precision for measurements.</p>
Precautions for measurement	
 <p>Perform measurement in upright position. Otherwise failure or low measurement precision may occur due to reagent leakage.</p>	 <p>Do not move the product during measurement. Otherwise measurement error may occur.</p>
 <p>Be sure to remove the reagent after measurement. Failure to do so may lead to liquid leak.</p>	
 <p>Do not use nor store the product in locations subject to large variations in temperature. Do not use nor store the product in locations directly exposed to wind from air-conditioning equipment. Allow the product to stand for 30 minutes or longer at room temperature before use when the product is moved from a hot or cold location. Otherwise measurement error may occur.</p>	
Caution on storage	
Store the product without condensation.	

## [About disposal]

Conform to disposal regulations established by your local government when disposing of the product and accessories.

## 3 Setup

### 3-1. Prepare the product

#### 3-1-1. How to attach the strap

Attach the strap as shown below.



#### Caution

- Do not swing the product holding the strap. Failure to do so may cause malfunction or poor precision for measurements.

#### 3-1-2. How to use the stand

Press "PUSH" to deploy the stand.



To close the stand, press the lower end of the stand to the original position.

#### Caution

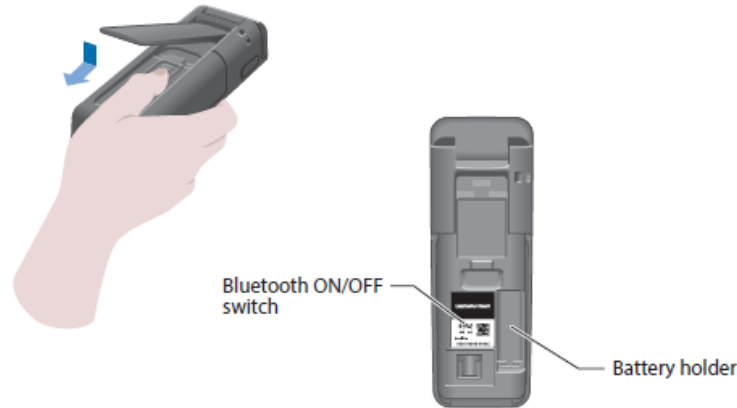
- Do not hold the product while the stand is open. Otherwise the stand may be damaged.



## 3-1-3. How to insert batteries

### To open/close the battery cover

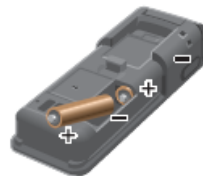
- ① Press "PUSH" to deploy the stand.
- ② Press the mark on the battery cover to remove the cover.



- ③ Attach the battery cover again.

### Place the batteries

Insert two new size AA alkaline batteries or two charged size AA nickel-hydride batteries, paying attention to polarities.



#### Replacement of batteries



5 seconds after remaining battery amount is displayed with low battery, the power is automatically turned off. Replace the batteries. After removing the batteries, the clock count continues for 60 seconds. Please replace the batteries within 60 seconds.

#### Caution

- Do not mistake the polarities of batteries.
- Do not mix batteries of different manufacturers or models, or new and used batteries.
- Follow the instruction manual for the batteries used.
- Size AA batteries back up the clock of the product. In cases when the batteries are dead or when the batteries are removed while the power is on, the clock may be initialized. In this case, adjust the clock.
- Conform to disposal regulations established by local governments when disposing of batteries.
- Remove the batteries when the product is to be stored for an extended period of time. Failure to do so may cause liquid leak and burst.

## 3-1-4. Bluetooth ON/OFF switching

Battery holder and Bluetooth ON/OFF switch are located inside the battery cover. The factory shipment setting of the Bluetooth ON/OFF switch is OFF.

To link the Application with a smartphone and/or a tablet, set the Bluetooth ON/OFF switch ON.

Connection with a smartphone, tablet, and/or PC allows automatic synchronization of time and date and timestamping of measurement data.

- ① If Bluetooth is not used, slide the Bluetooth ON/OFF switch to OFF. The Bluetooth is OFF unless Bluetooth ON is indicated on the display unit.



#### Caution

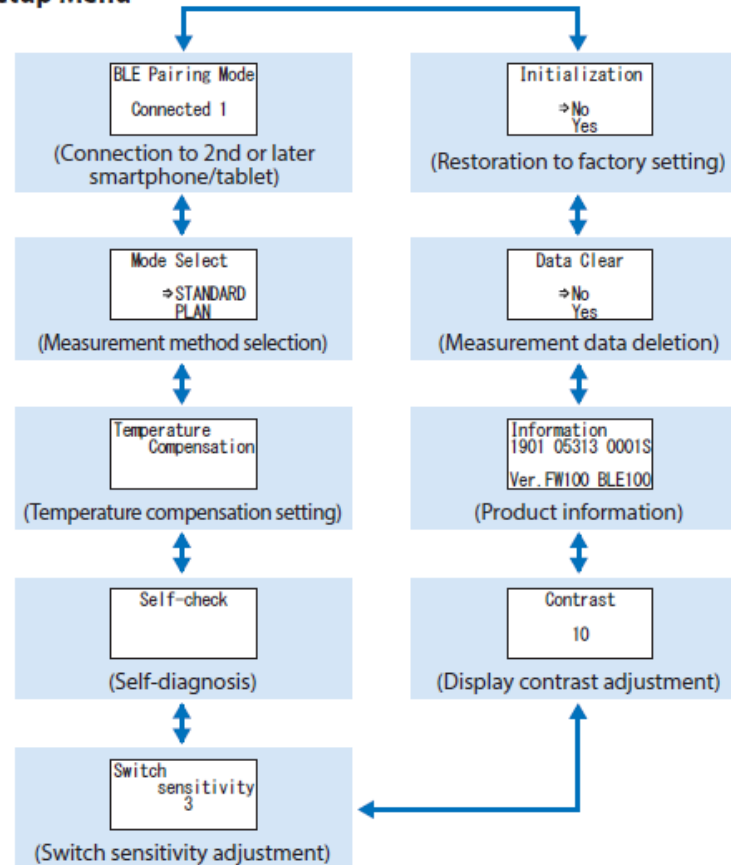
- Radio frequency bands and communication methods that can be used are defined for each country. In some countries, registration of wireless devices may be mandatory. Refer to the website below for countries where Bluetooth can be used.  
<https://biochemifa.kikkoman.co.jp/e/support/dl/smart>
- In a country not listed below, set the Bluetooth switch OFF.
- Bluetooth cannot be used to connect to a PC. Please use the provided USB cable.

## 6 Product Setup

You can configure the product settings.

- ① Press and hold the Power button for 5 seconds or longer and go to the setup menu.
- ② Pressing the Up or Down button switches the setting item.

### Setup Menu



\*\* Steps 4 & 5 of the Lumitester IFU manual have been omitted in this presentation as they are not relevant to the processing of 2 Min Water Control

- ③ Select a setup item and press START.
- ④ Pressing the Power button terminates the setup and the screen returns to the measurement screen.

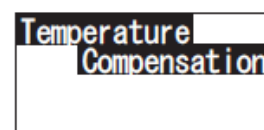
## 6-3. Temperature Compensation (Temperature compensation setting)

The reagent has a characteristic in which luminescence varies depending on the temperature.

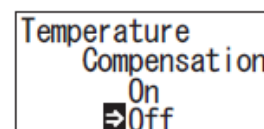
The temperature compensation is a function that corrects the temperature characteristic of the reagent by measuring the temperature of the product.

The temperature compensation is OFF by default.

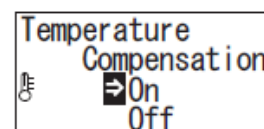
The range of the temperature compensation is +10 to +40°C.



- ① Press and hold the Power button for 5 seconds or longer and display the Setup screen. Then press Up/Down button to select "Temperature Compensation" and press START.



- ② Select availability of temperature compensation using Up/Down buttons, and then press START.



- ③ Selecting "On" displays the temperature compensation status.

- Even if the temperature compensation is ON, the error code "E047" or "E048" is displayed at the temperature 40°C or higher or 10°C or lower, respectively, during measurement countdown, and the measurement result without temperature compensation is displayed with the error "E".
- If the temperature compensation is ON, the measurement time at +10 to +13°C is 20 seconds.

### Caution

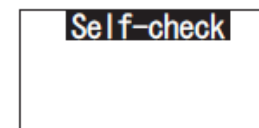
Allow the product and reagent to stand for 30 minutes or longer at room temperature before use.  
Do not use the product in locations subject to large variations in temperature. Failure to do so may cause poor precision for measurements.

## 6-4. Self-Check (Self-diagnosis)

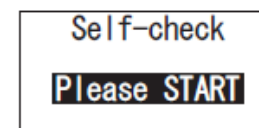
Keep the measurement chamber clean. Failure to do so may cause poor precision for measurements.

The self-check is a function to confirm the degree of contamination in the measurement chamber.

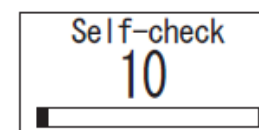
The temperature range of the self-check is +20 to +30°C.



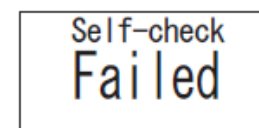
- ① Press and hold the Power button for 5 seconds or longer and display the Setup screen. Then press Up/Down button to select "Self-check" and press START.



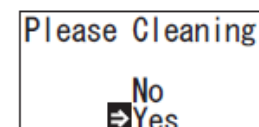
- ② Press START to start the self-check.



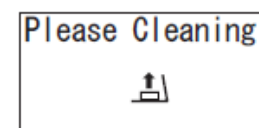
- ③ The check result is displayed after countdown. If "Passed" is displayed, the result is normal. Press START to return to the Setup screen.



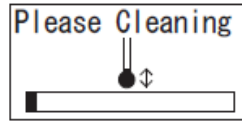
- ④ If "Failed" is displayed, clean the measurement chamber. Press START to go to the Cleaning screen.



- ⑤ To perform cleaning of the measurement chamber, press START then "Yes". If "No" is selected, the screen returns to the Setup screen.



- ⑥ Open the measurement chamber cover if closed.



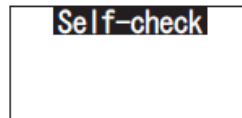
- ⑦ Opening the measurement chamber cover starts countdown and the interior of the measurement chamber emits light. Clean the measurement chamber.

## Maintenance of measurement chamber

1. Thoroughly wipe the locations that emit light in the measurement chamber with the provided cleaning brush soaked with ethanol.
2. Close the measurement chamber cover.

## Caution

- Do not pour ethanol into the measurement chamber.
- Do not apply ethanol to any part except for the measurement chamber.
- Do not use the product until it dries.



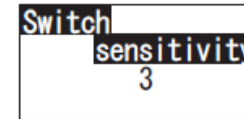
- ⑧ To make sure that the measurement chamber is clean, start the self-check steps from ① again.

## Caution

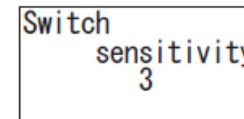
- If the temperature error is displayed, self-check is aborted. Turn the power off once, and allow the product to stand for 30 minutes or longer at room temperature before self-check.
- If "Failed" is still displayed even after cleaning, check the model and the serial number affixed inside the battery cover, and then contact the dealer or us.

## 6-5. Switch Sensitivity (Switch sensitivity adjustment)

You can adjust sensitivity of START and Up/Down buttons.



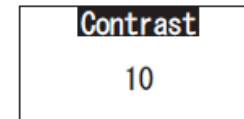
- ① Press and hold the Power button for 5 seconds or longer and display the Setup screen. Then press Up/Down button to select "Switch sensitivity" and press START.



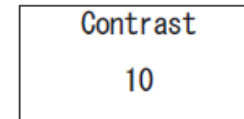
- ② Press Up/Down buttons to adjust the switch sensitivity. The adjustment range is 1 to 5. Pressing START adjusts the sensitivity and returns to the Setup screen.

## 6-6. Contrast (Display contrast adjustment)

You can adjust the contrast of the display panel.



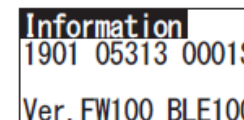
- ① Press and hold the Power button for 5 seconds or longer and display the Setup screen. Then press Up/Down button to select "Contrast" and press START.



- ② Press Up/Down buttons to adjust the contrast. The adjustment range is 1 to 20. Pressing START adjusts the sensitivity and returns to the Setup screen.

## 6-7. Information (Product information)

You can view product serial number, Software version (Ver.FW), and Bluetooth software version (BLE).



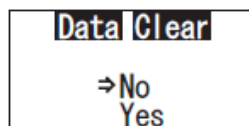
- ① Press and hold the Power button for 5 seconds or longer and display the Setup screen. Then press Up/Down button to select "Information".

# Lumitester Smart

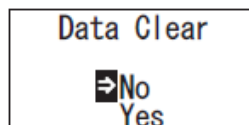
Cleaning & Maintenance

## 6-8. Data Clear (Measurement data deletion)

You can delete all data.



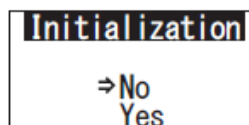
- ① Press and hold the Power button for 5 seconds or longer and display the Setup screen. Then press Up/Down button to select "Data Clear" and press START.



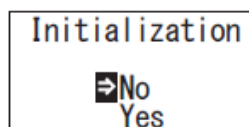
- ② Select Yes using Up/Down buttons, and press START. All the past measurement data will be deleted.

## 6-9. Initialization (Restoration to factory setting)

Time and date, past measurement data, and Bluetooth registration are deleted and the product is reset to the factory shipment status. The calibration data are reset to the factory shipment status.



- ① Press and hold the Power button for 5 seconds or longer and display the Setup screen. Then press Up/Down button to select "Initialization" and press START.



- ② Select Yes using Up/Down buttons, and press START.

The time and date cannot be set with this product alone. To set the time and date, an application-installed smartphone/tablet or software-installed computer must be connected.

The time and date can then be set automatically.



## Maintenance

### 7-1. Maintenance of the main body

If the product becomes contaminated, wipe with a dry cloth or soft paper such as tissue paper.

If the product becomes heavily contaminated, wipe with a soft cloth soaked with rubbing alcohol or diluted mild detergent solution.

#### Caution

- Do not spill liquids, reagents, organic solvents, etc. over the product. If any liquid is spilled on the product, wipe the liquid off immediately, remove the batteries, and allow the product to dry for 24 hours or longer indoors.



### 7-2. Maintenance of measurement chamber

If the reagent is spilled in the measurement chamber, clean the measurement chamber.

In addition, clean the measurement chamber approximately once every six months.

Perform self-check as needed. For self-check, refer to "6-4 Self-Check" (→P30).

- ① Turn the power off.
- ② Open the measurement chamber cover.
- ③ Thoroughly wipe the bottom and the side of the measurement chamber with the provided cleaning brush soaked with ethanol.
- ④ Close the measurement chamber cover.

#### Caution

- Do not pour ethanol into the measurement chamber.
- Do not apply ethanol to any part except for the measurement chamber.
- Do not use the product until it dries.



### 7-3. Replacement of batteries



5 seconds after the remaining battery level displays low battery, the power is automatically turned off. Replace with new batteries.

The clock count continues for 60 seconds after removing the batteries. Please replace the batteries within 60 seconds.

- ① Press "PUSH" to deploy the stand.
- ② Remove the battery cover.



- ③ Insert two new size AA alkaline batteries or two charged size AA nickel-hydrate batteries, paying attention to polarities.
- ④ Replace the battery cover.



#### Caution

- Do not mistake the polarities of batteries.
- Do not mix batteries of different manufacturers or models, or new and used batteries.
- Do not use an alkaline battery after its validity date has expired.
- Follow the instruction manual for the batteries used.
- Size AA batteries back up the clock of the product. When the batteries are dead or removed while the power is on, the clock may be initialized. In this case, adjust the clock.
- Conform to disposal regulations established by local governments when disposing of batteries.
- Remove the batteries when the product is to be stored for an extended period of time. Failure to do so may cause liquid leak and burst.

## 8

## Troubleshooting



### Warning



When abnormality is observed, turn off the power immediately, and remove the batteries as soon as possible.

Disconnect the USB cable if being used, and then remove the batteries.



When abnormality is observed such as malfunction, burning smell, fuming etc., there is a danger of fire and burst. Make sure that fumes are extinguished, and contact the dealer or us. Never repair the product by yourself, as this is very dangerous.

Error code displays for indicating operation mistakes and problems.

## 8-1. List of Error Codes

The details of Error codes and countermeasures are described below.

When the error code is still displayed after countermeasures are taken, check the model and serial No. affixed inside the battery cover, and contact the dealer or us.

Error codes	Details	Countermeasures
<b>E011</b> ***** E011 to 019 Memory error	Shows error in memory data that might be caused by the removal of batteries while memory data is written or read.	Turn the power off once, and then turn it on again. If the same error is still displayed, perform "6-8. Data Clear" (→P33).
#0004 <b>E021</b> E021 to 029 Measurement error	Shows error in measurement that might be caused under locations subject to large variations in temperature.	Turn the power off. Allow the product to stand for 30 minutes or longer at room temperature before use when the product is moved from a hot or cold location.
	Shows error in measurement that might be caused under locations such as exposure to direct sunlight.	Turn the power off, and move the product to a location not exposed to direct sunlight.
	Shows error in measurement that might be caused under locations such as loose closure of chamber cover.	Close the measurement chamber cover securely.
<b>E031</b> E031 to 039 Calibration error	Shows error in calibration that might be caused under locations subject to large variations in temperature.	Turn the power off. Allow the product to stand for 30 minutes or longer at room temperature before use when the product is moved from a hot or cold location.
	Shows error in calibration that might be caused under locations such as exposure to direct sunlight.	Turn the power off, move the product to a location not exposed to direct sunlight.
	Shows error in calibration that might be caused under locations such as loose closure of Chamber cover.	Close the measurement chamber cover securely.
<b>E041</b> XX.X°C E040 to 049 Temperature error	Shows error in temperature that might be out of range.	Turn the power off, and move the product to a location with a temperature of +5 to +40°C. When temperature compensation is ON, use the product within the temperature range (+10 to +40°C).
	Shows error in temperature that might be caused under locations subject to large variations in temperature.	Turn the power off. Allow the product to stand for 30 minutes or longer at room temperature before use when the product is moved from a hot or cold location.
<b>E051</b> E051-059 Product error	Shows error in product that might be caused by malfunctions of electric parts.	Turn the power off once, and then turn it on again.

## 8-2. Other problems and countermeasures

The details of problems, causes, and countermeasures other than those for error displays are described below.

When the normal condition is not recovered after the countermeasure is taken, when any problem other than these occurs, or when requesting repairs, check the model and the serial No. affixed inside the battery cover, and then contact the dealer or us.

Details	Possible causes	Countermeasures
Power cannot be turned on.	Batteries are not inserted. Batteries are drained.	Insert new batteries. Refer to "7-3. Replacement of batteries" (→P35).
Power cannot be turned off.	The product is not operating normally due to a malfunction of electronic parts.	Reinsert the batteries again.
	The USB cable is connected. Key operation is not accepted while the product is connected to a PC.	Exit the control software, and then disconnect the USB cable.
Power is automatically turned off.	Batteries are drained.	Insert new batteries. Refer to "7-3. Replacement of batteries" (→P35).
	When the product has not been operated for 10 minutes, the power is automatically turned off.	This is not a malfunction.
Measured values seemed to be lower.	The measurement chamber is contaminated.	Perform "7-2. Maintenance of measurement chamber" (→P34).
Water hazard.	The surface of the product was exposed to water.	Turn the power off immediately and wipe away water from the product. Detach the battery cover, remove the batteries, and dry the product with the operation panel turned upward and the measurement chamber cover opened. Allow the product to stand at room temperature for approximately 24 hours.
	Water has entered the measurement chamber.	Turn the power off immediately and remove the reagent. Wipe off water with the cleaning brush, and dry the product with the operation panel turned upward and the measurement chamber cover opened. Allow the product to stand at room temperature for approximately 24 hours. Refer to "7-2. Maintenance of measurement chamber" (→P34).



## 9

## Specifications

Name	Lumitester Smart
Detecting method	Integration employing a photodiode
Dark noise	10 RLUs or less
Detection reagent	Dedicated disposable type
Measurement range	0 to 999999 RLUs
Temperature compensation range	+10 to +40°C
Measurement time	10 seconds
Display	Organic LED
AUTO ZERO calibration	Built-in
Auto power-off	10 minutes
Clock	Built-in: date and time Valid only when the Application is connected (automatic synchronization)
Measurement data	RLU
Interface	USB, Bluetooth LE (Ver.4.1)
Number of memory data points	2000
Ambient temperature range	+5 to +40°C
Ambient humidity range	20 to 85%Rh (free from condensation)
Storage temperature range	-10 to +50°C
Storage humidity range	20 to 90%Rh (free from condensation)
Power supply	Two size AA alkaline batteries or two size AA nickel-hydrate batteries
Dimensions	Approx. 65mm (W) x 176mm (H) x 40mm (D)
Mass	Approx. 235 g (without batteries)

## 10

## External View



# 2 Min Water Control

What's inside the box

**NOVEO**  
REAGENTS

2 MIN REAGENT KIT  
12 ANALYSIS

FOR USE WITH  
KIKKOMAN LUMITESTER SMART

**NOVEO**  
2 MIN WATER CONTROL  
REAGENTS

## 2 Min Water Control

2 Min Water Control Accessories  
Packaging - Front



2 Min Water Control  
Reagent Kit  
Packaging - Front

2 Min Water Control Accessories  
Packaging - Back



2 Min Water Control  
Reagent Kit  
Packaging - Back

## 2 Min Water Control

3 x aluminium foil packs of Substrate  
Each contains 4 x Lumitube with lyophilised enzyme



Certificate of Analysis



2 Min Water Control  
Instruction Leaflet



2 Min Water Control  
Reagent Kit Packaging



1 x bottle EXTRACTANT  
1 x bottle STANDARD 1000



12 x 0.45µm sterile  
syringe filters



12 x Sampling Jars



12 x 50ml Sterile Luer  
Lock Syringes

# **Lumitester Smart & 2 Min Water Control**

Set up for water testing

## All the components to Start Testing



## Measurement procedure



- ① Press and hold the product's Power button for a while.  
"Lumitester" is displayed and it is ready for measurement after countdown.



### Caution



When the buzzer sounds with the reagent warning blinking, open the measurement chamber cover and remove the reagent.



When the buzzer sounds with the measurement chamber cover warning blinking, close the measurement chamber cover.

# **Lumitester Smart & 2 Min Water Control**

Processing a Sample



## Quantification of total bacteria in Dental Unit Water by ATP-metry.

After running water lines on dental unit for 1 minute, collect approximately 50ml of water from the line(s), using the sampling container.

Reagents stored at room temperature and in the dark have a **3-month** shelf life.

**To preserve 12-month shelf life, reagents should be refrigerated (2- 8°C).**

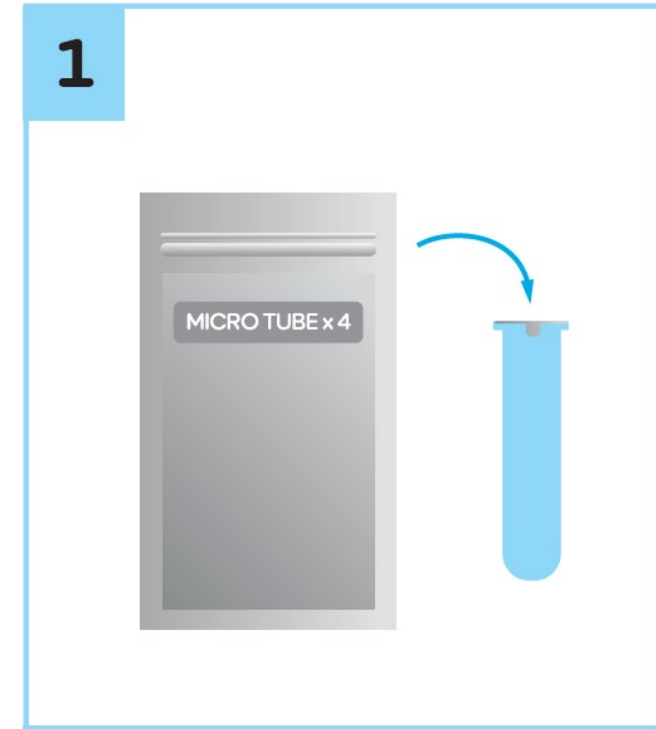
Take the dropper bottles of **EXTRACTANT** and **STANDARD 1000** as well as a **microtube** (lyophilized enzyme) from the refrigerator.

Items must be at room temperature (above 18°C) before use.

Prepare plastic consumables (sampling container, syringe and bacterial filter) and turn on the Lumitester Smart.

### Phase 1: installation

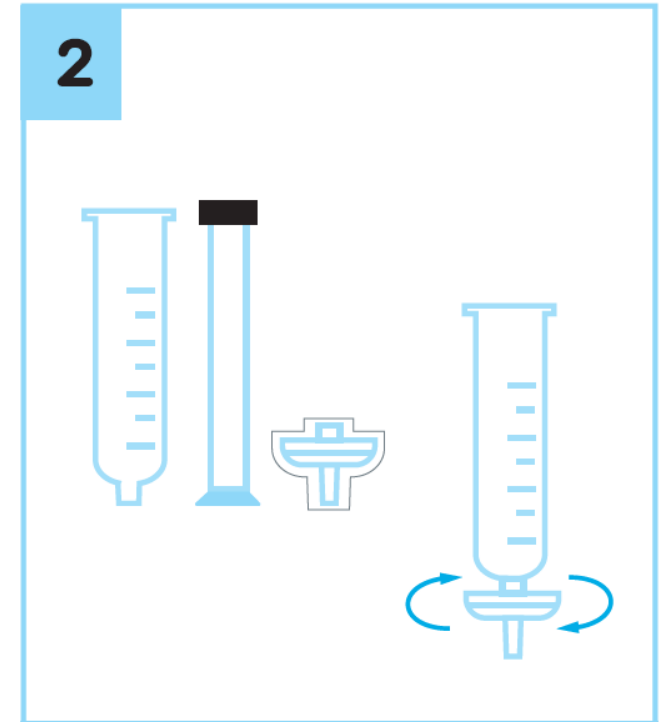
- On a flat and clean surface, prepare the luminometer, a dropper bottle of 2 Min **EXTRACTANT** and **STANDARD 1000** with all the necessary consumables.
- Turn on the luminometer (measurement chamber closed) and wait 10 seconds for the device calibration.
- Take one Micro tubes (Lumitube) from the aluminium pack. Remove aluminium foil seal and place tube on a tube rack for support.



At this moment, the reagents 2 MIN REAGENT<sup>®</sup> and STANDARD 1000 must be at room temperature (between 18°C and 25°C) to ensure maximal efficiency of the enzyme.

### Phase 2: quantification of the total flora in a water sample

- The first step aims to filtrate the water sample to concentrate the microorganisms on the filter (0.45µm pore size).
- Take the syringe out of its package being careful not touch the nozzle
- Remove the syringe piston. Do not touch the Teflon part with hands or contaminated instruments
- Remove the packaging seal of the filter but do not remove the filter from the plastic packaging. With the filter still in the cup package, screw the syringe onto the filter. Keep the filter packaging for later.



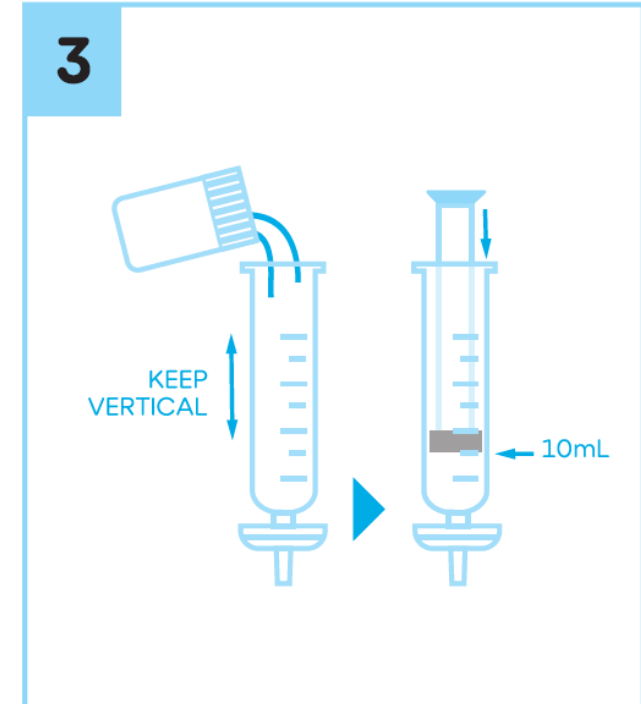
*From this stage, the following steps must be performed in a short space of time to obtain an optimal result.*

## Phase 2 (continue): quantification of the total flora in a water sample

- Pour the sample vial content (50 ml or less) into the syringe (which is now screwed to the filter),
- Write down the volume filtered,
- Insert the piston inside the syringe and filter all the sample until the filter grooves are visible once again. Stop pushing to avoid damage to the membrane.
- Make sure the reagent **EXTRACTANT** is at room temperature ( $\sim 18^{\circ}\text{C}$ ) and place 4 drops of **EXTRACTANT** in the base of the plastic packaging the filter came in.



*From this stage, the following steps must be performed in a short space of time to obtain an optimal result.*

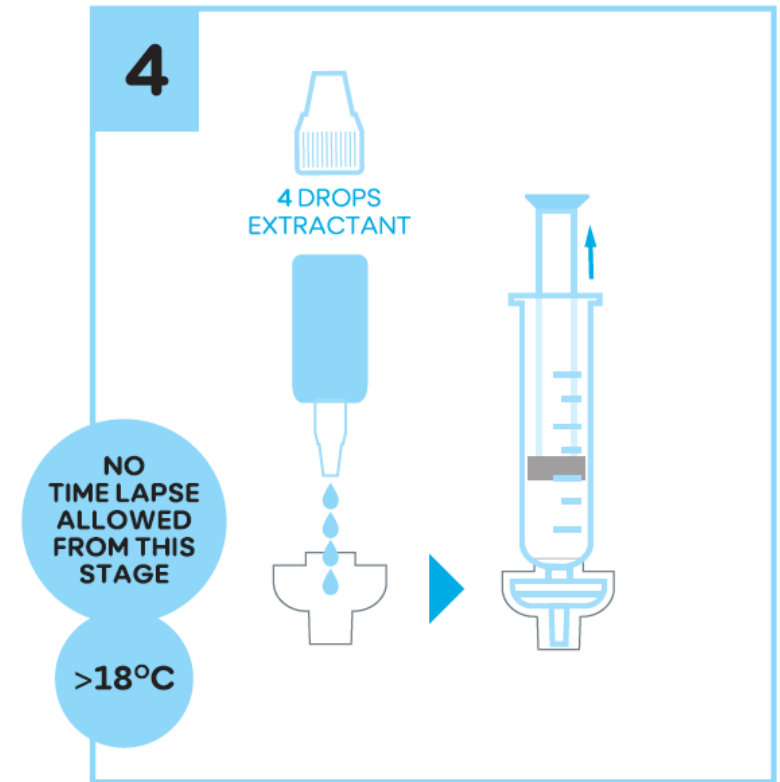


### Phase 2 (continue): quantification of the total flora in a water sample

- Make sure the reagent **EXTRACTANT** is at room temperature ( $\sim 18^{\circ}\text{C}$ ) and place 4 drops of **EXTRACTANT** in the base of the plastic packaging the filter came in.
- Draw up all the **EXTRACTANT** through the filter. Maintain depression inside the syringe.



*From this stage, the following steps must be performed in a short space of time to obtain an optimal result.*

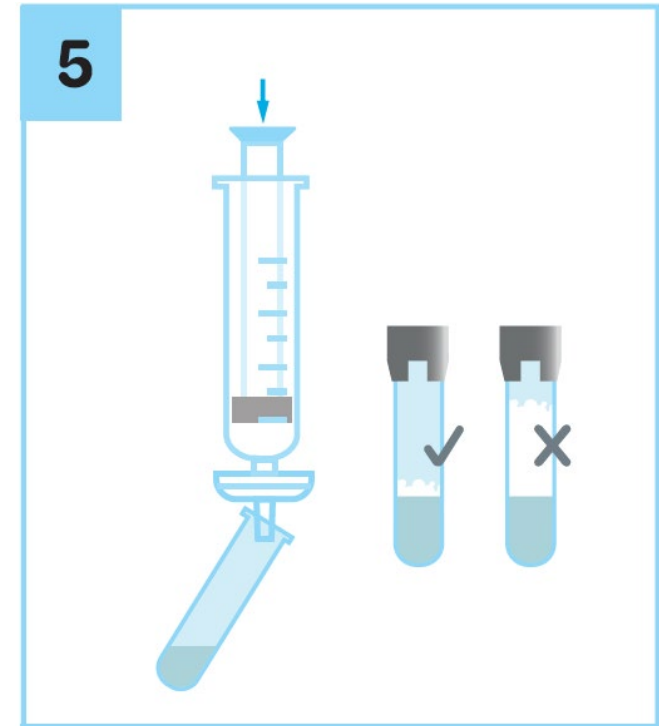


### Phase 2 (continue): quantification of the total flora in a water sample

- With firm and constant pressure, push the liquid out of the syringe into the micro tube until a thick foam comes out. Avoid an excess of foam in the micro tube.
- Stop pressure as soon as foam appears to avoid creating <stopper> in the upper part of tube.



*From this stage, the following steps must be performed in a short space of time to obtain an optimal result.*



### Phase 2 (continue): quantification of the total flora in a water sample

- Fix the micro tube in the tube holder
- After fixing microtube onto holder, agitate solution for 15 secs to homogenize
- Place them in the luminometer and press the ENTER button
- Write down the R1 result in RLU (Relative Light Unit)



*From this stage, the following steps must be performed in a short space of time to obtain an optimal result.*

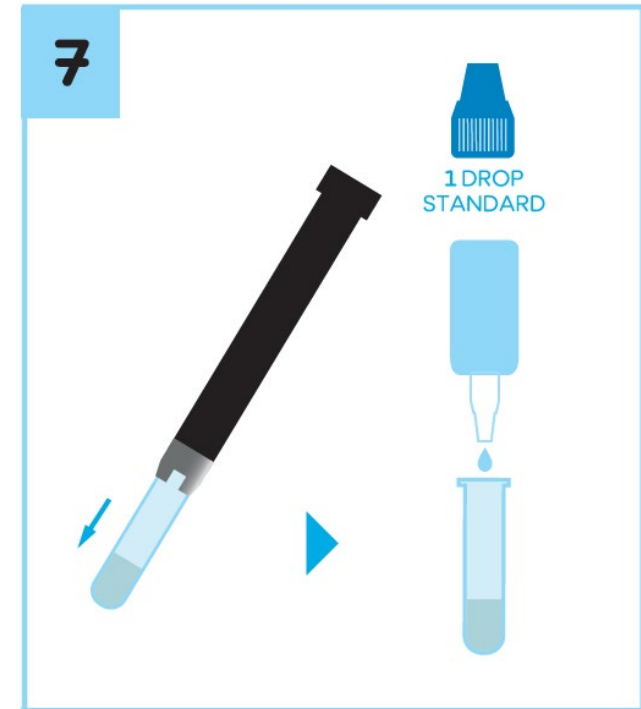


### Phase 2 (continue): quantification of the total flora in a water sample

- Open the cover and retrieve the micro tube
- Add one drop of **STANDARD 1000** into the micro tube, without inserting nozzle of the dropper bottle to avoid contact with the micro tube. In case the foam forms a barrier in the upper part of the tube, tap the tube on a flat surface to get the foam down.



*From this stage, the following steps must be performed in a short space of time to obtain an optimal result.*





### Phase 2 (continue): quantification of the total flora in a water sample

- Fix the micro tube in the tube holder
- Homogenize the mix by tapping the micro tube on a flat surface
- Place it in the luminometer and press the ENTER button
- Write down the R2 result in RLU (**Relative** Light Unit)
- Enter the data into the Excel file



*From this stage, the following steps must be performed in a short space of time to obtain an optimal result.*



## 2 Min Water Control Monitoring



EQUIVALENT BACTERIA OR CFU/ml CALCULATION AFTER ATP DOSAGE IN RLU FROM FILTERED WATER SAMPLE  
(in eq.bact./ml or CFU/ml)

2 minutes Unit-Oral : Biomonitoring with Lumitester Smart

Measurement Date	Sample Id.	Volume analysed [1-60] (in ml)	Sample Result = R1 (in RLU)	Result after standard addition = R2 (in RLU)	Quality Control Validation	Sensitivity (RLU/pg)	ATP Concentration (in pg/ml)	Equivalent Bacteria / ml or CFU/ml	Quality Control troubleshooting
Example	1	50	11	1271	Validated	1.26	0.17	175	
Example	2	51	41	1441	Validated	1.40	0.57	574	
Example	3	50	0	143	Failed	0.14	0.00	0	The reagent DENDRIDAG SW is too cold. Warm up the tube to 20°C-25°C or the reagent DENDRIDAG SW is no longer active (out-of-date or degraded)
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
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					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	
					Validated	0.00	#DIV/0!	#DIV/0!	

The Excel file gives the total biomass concentration for each sample in picogram ATP per millilitre, equivalent number of bacteria per millilitre and the logarithm of equivalent number of bacteria per millilitre.

# **2 Min Water Control**

Troubleshooting guide

### Measurement anomaly

During measurement, it is possible that the standardisation is not correct. The Excel file will automatically warn you in case this anomaly is detected after filling the grey columns. Immediately get the test tube out of the luminometer, homogenise the mix and restart the measurement. If the problem continues, several possibilities should be considered:

- There is too much foam in the upper part of the tube that prevent the **STANDARD 1000** from mixing with the sample. Restart the complete protocol avoiding an excess of foam and making sure the tube is correctly homogenized.
- The reagent **EXTRACTANT** is too cold. Warm up the tube to 20°C-25°C.
- The reagent **EXTRACTANT** is no longer active (out-of-date or degraded). Restart the complete protocol using a new bottle of **EXTRACTANT**.
- The sample analysed has an inhibitory effect on the enzyme activity of the reagent **EXTRACTANT**. Restart the complete protocol filtrating a smaller volume of water and rinsing the filter with sterile water.
- The luminometer may not be functioning properly (e.g., dirty measuring cell, faulty shutter mechanism, or malfunctioning photodiode). Please check the luminometer's response using a Kikkoman calibrator pen.

## Controls

### ***Control of the luminometer contamination***

#### *a) Test:*

- Fix an empty test tube to the tube holder,
- Place it in the luminometer and press the ENTER button,
- The result should be less or equal to 1 RLU.

#### *b) In case of contamination, follow this procedure:*

With a cotton swab, wipe the internal surfaces of the measurement chamber.

### ***Control of the reagent's contamination***

#### ***a) Test:***

- In a test tube, put 2 drops of **EXTRACTANT**
- Fix test tube to the tube holder
- Place it in the luminometer and press the ENTER button
- The result should be less or equal to 5 RLU

#### ***b) In case of contamination, follow this procedure:***

Discard the contaminated reagent and select a new bottle of **EXTRACTANT**.

### *Control of the reagent's efficiency*

#### *a) Test:*

- In a test tube, put 2 drops of **EXTRACTANT** and 1 drop of **STANDARD 1000**
- Homogenise the tube,
- Fix the test tube to the tube holder,
- Place it in the luminometer and press the ENTER button,
- The result should higher than 200 RLU.

#### *b) In case of an efficiency loss of RLU signal, follow this protocol:*

Discard the contaminated reagent and select a new bottle of **EXTRACTANT**.

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