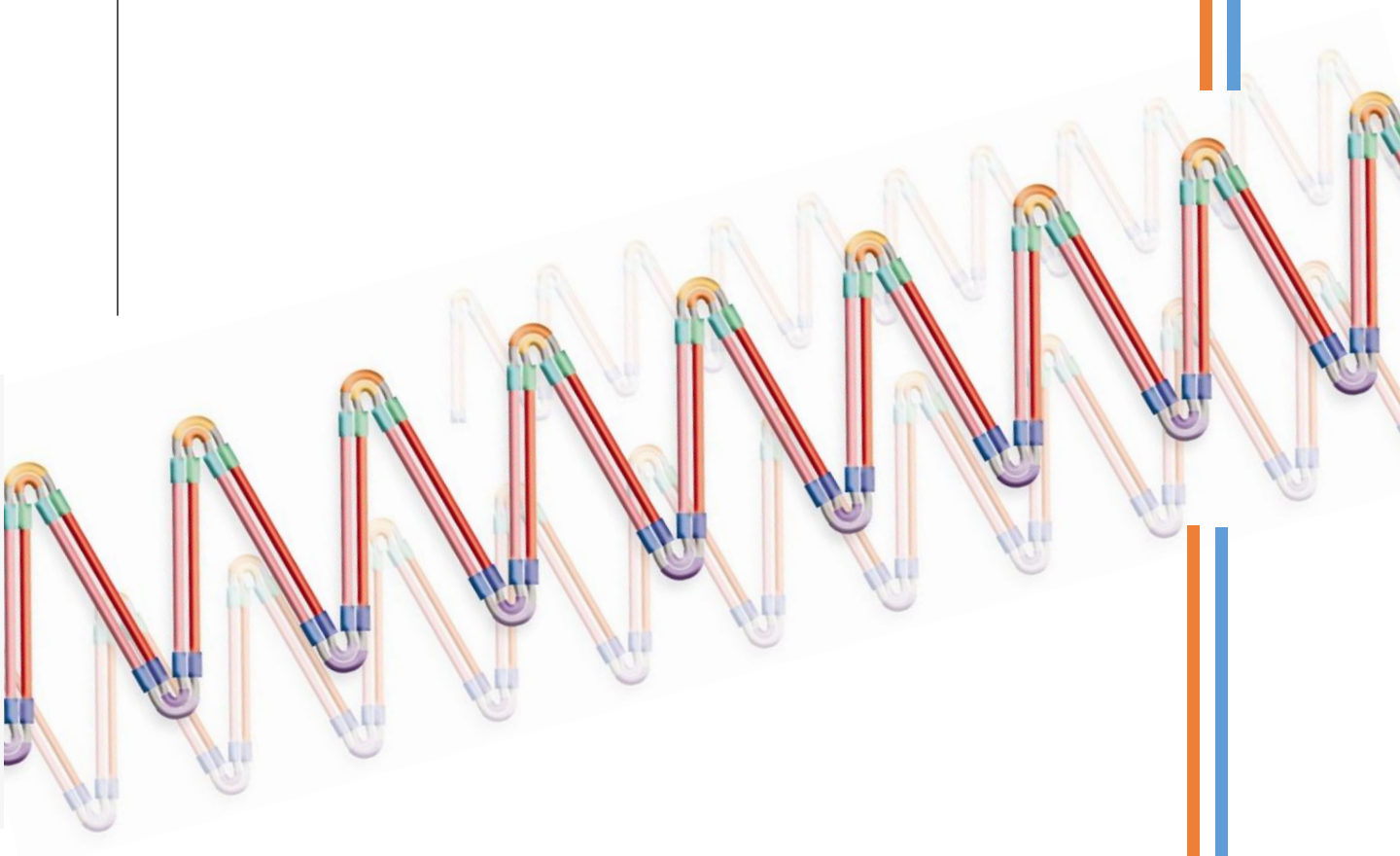
Loopamp®

LAMP (Loop-mediated Isothermal Amplification) method

'Eiken'

LAMP Operation Guide



EIKEN CHEMICAL

Table of Contents

0. Scope of LAMP Operation Guide

1. Scope ------ P.1

I . Precautions of LAMP operations

1. Introduction ------ P.2
2. Warning and precautions ------ P.2
3. Precautions during operations ------ P.7
4. What to do when contamination is suspected ------ P.9

0 Scope of LAMP Operation Guide

1. Scope

The LAMP Operation Guide describes the requirements to be complied by the laboratories offering LAMP test of Eiken Chemical. The guideline, requirement and performance criteria outlined in this document are intended for comparable, accurate and reproducible results.

For more details, refer to the Instructions for Use (IFU) for each Loopamp™ product.

I Precautions of LAMP operations

1. Introduction

The LAMP reaction is very sensitive and poses a risk to lead to erroneous results if samples are contaminated with even a trace amount of amplified products or specimens.

There are two types of contamination as follows:

- Cross contamination ⋯ The contamination by the test environment, between specimens and positive control.
- Carry-over contamination ⋯ The contamination with amplified products.

To avoid such contamination, please check and follow precautions before starting operation.

2. Warning and precautions

Basic precautions

◇ Wash your hands before/after the operations.

◇ Put personal protective equipment (PPE) on before starting:

- Disposable face mask
- Gloves : - Disposable powder-free gloves.
  - Use a proper size.
  - Put on double gloves.
  - Take off the first pair of gloves or change to a new pair of gloves before you move to other workspaces.
- Safety goggles : Wear goggles when required/necessary.

◇ Avoid personal talk during the operation and forbid drinking, eating and smoking in a laboratory.

NOTE : DNA-degrading enzymes (DNase) and RNA-degrading enzymes (RNase) are in skin and body fluids such as sweat, saliva and blood. Pay attention to avoid letting them contaminate the workspaces.

Things to do before/after operation

◇ Cleaning workspaces and equipment using 0.5% sodium hypochlorite solution.

[Procedure]

・Workspaces

① Distribute and spread 0.5% sodium hypochlorite solution with a paper towel and wait for 5 minutes.
② Wipe off the liquid and wipe with a paper towel wet with water.
③ Wipe up by a dry paper towel.

・Equipment (Pipettes, an ice rack, etc.)

① Wipe surfaces with a paper towel wet with 0.5% sodium hypochlorite solution and wait for 5 minutes.
② Wipe off the liquid and wipe with a paper towel wet with water.
③ Wipe up by a dry paper towel.

NOTE : Ventilate well when you use sodium hypochlorite solution.

Sodium hypochlorite solution (Bleach)

・To avoid contamination, use hypochlorite solution to degrade DNA and RNA. Meanwhile, alcohol cannot degrade them.

・Use 0.5% sodium hypochlorite solution for usual cleaning since a higher concentration of it may damage metal. If contamination occurs, use 2% sodium hypochlorite solution. Also, if you use it on metal, wipe up by a wet paper towel or spread distilled water and wipe them up by a paper towel.

・Store undiluted sodium hypochlorite solution in a cool and dark place since it would be decomposed by light or heat. After 1 week from the date you made, replace the old one with new 0.5% sodium hypochlorite solution.

※ Sodium hypochlorite:
Concentration of household bleach (ex: 5%)

⇒ 0.5 % sodium hypochlorite・・・Dilute 10 times by distilled water
⇒ 2 % sodium hypochlorite・・・Dilute 2.5 times by distilled water

✓ How to know degradation of a sodium hypochlorite solution.

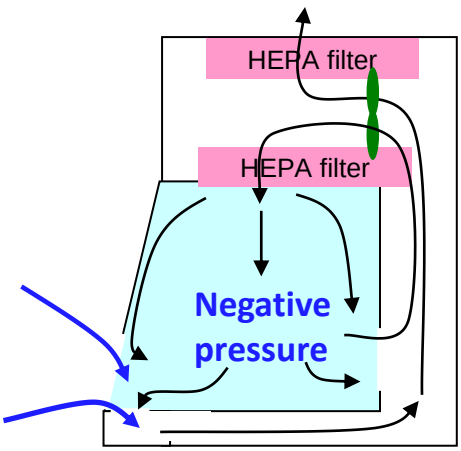
・Change of the color from yellowish color to non-color.

NOTE : Mark the date of dilution on a wash bottle. Discard it and make again after 1 week passed the date of dilution.

Biosafety cabinet and clean bench

[Biosafety cabinet]

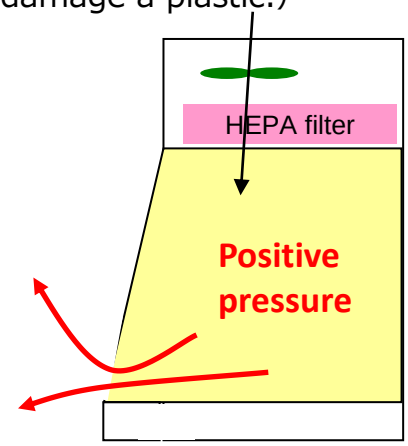
- Equipment to prevent contamination from inside to outside of equipment.
- Ventilate clean air through the HEPA filter (high efficiency particulate air filter) and create negative pressure in equipment.
- There are three types of biosafety cabinets which comply with biosafety level Ⅰ toⅢ, and you should select the proper level of cabinet as per the microorganisms, etc. which you will handle.
- Handle specimens in a biosafety cabinet during nucleic acid extraction if available.



Where applicable, follow your laboratory rules regarding how to handle infectious microorganisms.

[Clean bench]

- Equipment to prevent contamination from outside to inside of equipment.
- When doing aseptic operation, take air in through HEPA filter and keep positive pressure in the equipment.
- The reagent preparation should preferably be done in a clean bench.
- Sterilize inside of the clean bench by UV light after operation.
  (NOTE: UV light may damage a plastic.)



Workspace

To avoid nucleic acid contamination, separate workspaces for each procedure. The classification of workspace is determined by a concentration of nucleic acid and a grade of biological risk.

【Workspace and Procedure】

Reagent preparation

Workspace A (Optional*)

【Nucleic acid concentration: None】
Low-contamination risk of nucleic acid and low biohazard risk

- ◆ A clean bench
- ◆ A designated laboratory for reagent preparation
- ◆ A designated workbench for reagent preparation

*You may or may not prepare this area depending on the size of the available workspaces.

Specimens handling and extraction

Workspace B

【Nucleic acid concentration: Low-Medium】
An area for preventing infection from a specimen, with low-medium contamination risk of nucleic acid but high-biohazard risk.

- ◆ A biological safety cabinet
- ◆ A designated laboratory for handling specimen
- ◆ A designated workbench for handling specimen

Amplification reagent and mixing extracted sample

Workspace C

【Nucleic acid concentration: Medium】
A contamination risk of nucleic acid is somewhat higher than the Workspace B as positive control contains high concentration of the target templates, but a biohazard risk is lower than the Workspace B.

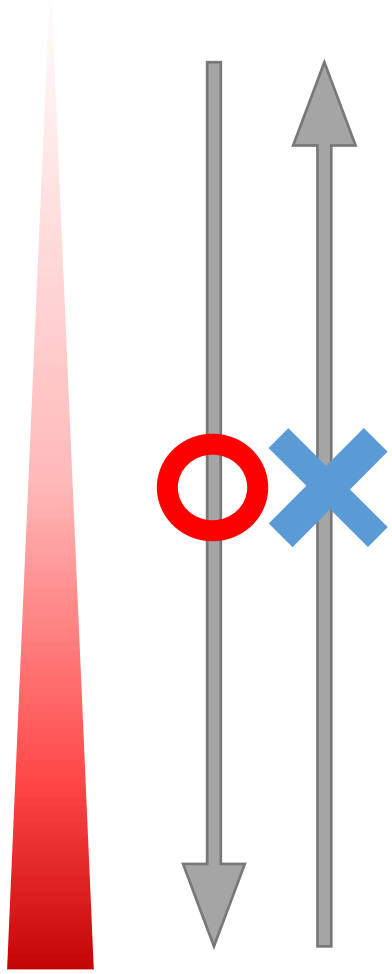
Amplification (Where to install an amplifier)

Workspace D

【Nucleic acid concentration: high】
An area which must be strictly separated from other areas as it poses the highest risk of nucleic acid contamination.

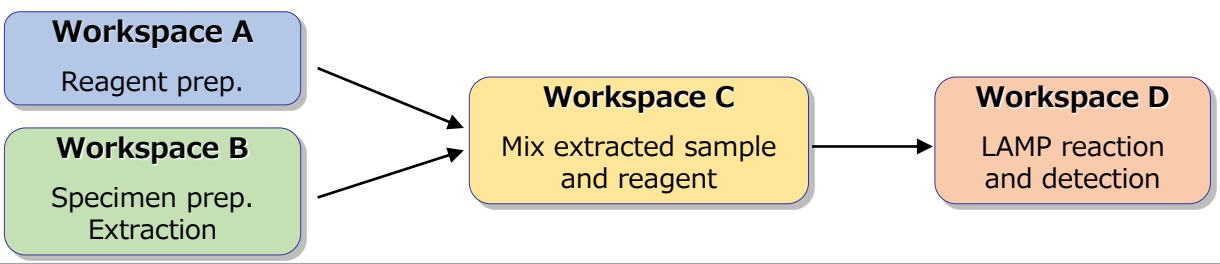
- ◆A laboratory where an amplifier is installed.

【Nucleic acid concentration】 【Transport of tools within a facility】



- Separate workspaces depending on a concentration of nucleic acid you will handle. The concentration of nucleic acid will increase as operation progress.
- As the general rule, the transport of tools within a facility should be Workspace (A→)B→C→D (The concentration of nucleic acid: Low→High). The opposite direction is prohibited.

<Operations and Workspaces>

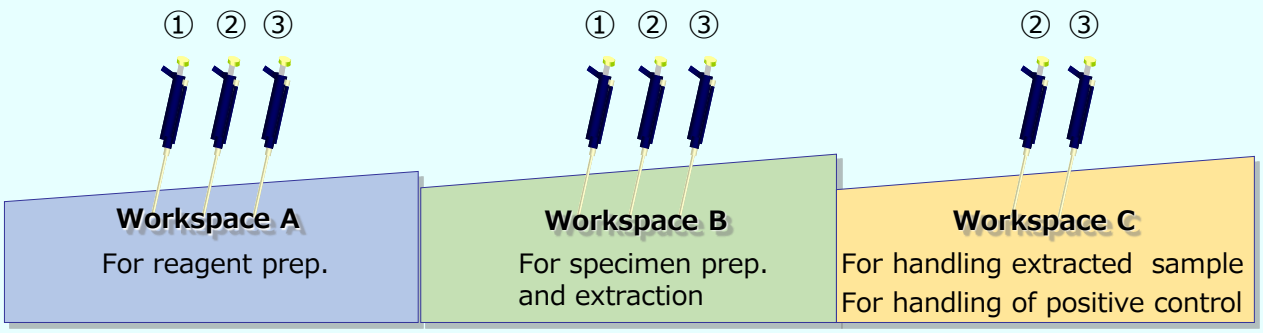


◇ Equipment

Equipment (micropipettes, filter pipette tips, a tube rack, etc.) should be used in each designated workspace. Use those equipment with putting gloves on and discard/change gloves when you move to other workspaces.

- Disposable microtubes: sterilized disposable microtubes. Discard it after you used.
- Pipette tips : Sterilized disposable pipette tips with hydrophobic filter of DNase and RNase free. Discard it after you used.

<Example of workspaces when use micropipettes>



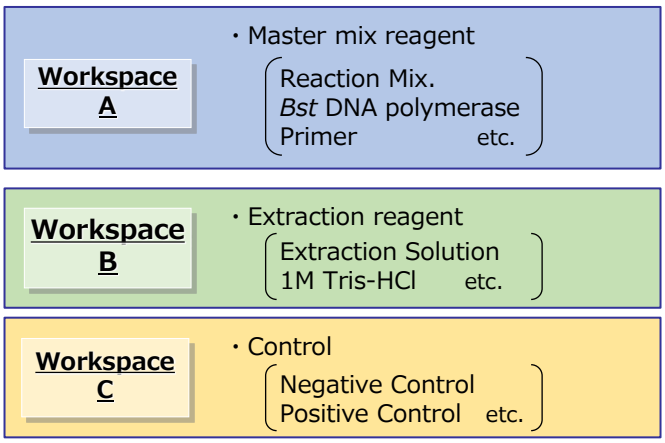
① 200～1000 µL micropipette
② 20～200 µL micropipette
③ 2～20 µL micropipette

※The type and the number of micropipettes depend on the process of operations.

◇ Reagent

Use each reagent at each designated workspace.

<Example of workspaces when you handle reagent>



3. Precautions during operations

◇ Micropipette

- Use a disposable filter tip and do not reuse it.
- Please make sure the tip of a filter tip does not touch anything. Organize the work environment to avoid the tip of a filter tip touching unexpected things.
- If your hand or something touches the tip of a filter tip, change the filter tip.
- Pipette slowly to prevent splattering liquid.
- Make sure that the micropipette body itself does not touch inside of a sample container or a trash bin for filter tips.

◇ LAMP reaction tubes

- Do not touch inside of the lid of a rection tube since dried amplification reagent is attached.
- Do not put your hand inside the aluminium bag to avoid contamination. Take a container of reaction tubes out by pushing the other end of an aluminium bag.
- Close the lid of the reaction tube one by one immediately after filling it.

◇ Heating process

- Use a heating block. A water bath may cause contamination.
- When heating 1.5 mL tubes, use cap-locks to avoid opening the lids of the tubes.

◇ Positive control

- When dispensing positive control into reaction tubes, follow below process: Sample solution → Negative control → Positive control. Dispense positive control at last to avoid contamination.
- Spin down or flick down a tube of positive control before opening.
  → It might have contamination risk if positive control liquid remains on (the inside of) the lid of the positive control tube and splatters when opening the lid.
- To prevent contamination in a workspace, do not use positive control except the intended use mentioned in the Instructions for Use.

◇ If extracted sample solution or positive control is spilled

① Do not wipe off, otherwise extracted sample solution or positive control will spread. Put a paper towel and pour sodium hypochlorite solution on it and wait for 5 minutes.

② Discard a paper towel and pour sodium hypochlorite solution again onto the wet area. Wait for another 5 minutes.

③ After 5 minutes, wiping off sodium hypochlorite solution and wipe again with distilled water. Then, wipe again with a dried paper towel.

◇ How to handle amplified products

- Do not open the lid of the reaction tube after the LAMP reaction.
- Do not perform electrophoresis of amplified products.
- Do not bring amplified products into other workspaces except Workspace D.

◇ Disposal

[Reaction tubes]

- Do not open the lid of the reaction tube after the LAMP reaction.
- Take reaction tubes carefully from an instrument to avoid opening the lids of reaction tubes.
- Keep the lids of reaction tubes closed and put them into double plastic zipper bags which can be incinerated and shealed. Also, follow your laboratory/local regulation.
- To prevent amplified products from spreading, do not autoclave the reaction tubes.

[Others]

- Follow your laboratory/local regulation.

4. What to do when contamination is suspected

Suspected result of contamination

◇ If you get a result like followings, there is possibility of contamination:

- The result of negative control shows positive.

How to check whether contamination occurs

Try LAMP reaction again with negative control and positive control, and if you get the result for negative control positive again, then it has possibility of contamination. (If you get positive result for negative control only the first time, misdispensing cannot be ruled out.)

Cause of contamination and how to reduce the risk

◇ The cause of contamination cannot be identified immediately, but there are two main possibilities as follows:

① A positive sample or extracted solution of a positive sample is mixed into another tube.
⇒In this case, if you pay attention to the operation between samples, this will not occur again.

② DNA spilled over to workspaces or equipment enter reagents or reaction tubes. (Possible causes are a positive sample, a positive control and amplified products.)
⇒In this case, a large scale of cleaning for all workbenches, equipment and laboratory is required. Especially in the case that LAMP amplified products was the cause of contamination, work-environmental cleaning is essential since it is highly possible that a large amount of DNA is diffused.

What to do against contamination

【 Cleaning 】

Use 2% sodium hypochlorite solution.*

NOTE : Ventilate well when using sodium hypochlorite solution.

* Regarding the daily cleaning, use 0.5% sodium hypochlorite solution.
  However, 2% concentration is required when contamination is suspected.

<Where and what to clean>

① Workbench, desk, chair, biosafety cabinet (and clean bench), etc.

② Tube rack, ice rack, etc.

③ Micropipette.

④ LAMP reaction instrument, heat block, centrifuge.

⑤ Anywhere you touched by hands such as doorknob, etc.

【 Disposal 】

Discard all consumables if possible. If you leave consumables in use, they may cause contamination.

ex : Reagent, LAMP reaction tubes, 1.5 or 2.0 mL microtubes,
filter tips with a container, etc.

【 Check cleaning efficiency 】

Check the efficiency of cleaning with using unopened reagent and make LAMP reaction on N=3 of negative control and N=1 of positive control at workspaces and equipment you cleaned. If all negative control does not show positive, it would be recognised that the cleaning was done sufficiently.

What and how to do against contamination when suspected

【Workbench, desk, chair, biosafety cabinet (and clean bench), etc.】

① Wipe thoroughly with 2% sodium hypochlorite solution using a paper towel. For a safety cabinet (and a clean bench)[*1)], wipe tables and surfaces of glass as well as areas touched by hands (switches, etc.) Also, wipe all areas you touched such as shelves, drawers, refrigerators, doorknobs, etc.

② Wait for 5 minutes, and wipe off water with a paper towel.

③ Wipe thoroughly with water (tap water is also acceptable) using a paper towel.

*1) Irradiate the safety cabinet (and the clean bench) with UV after cleaning (approx. overnight).

【 Tube rack, ice rack, etc.】

Soak equipment[*2)] as follows if possible.

① Soak equipment in 2% sodium hypochlorite solution completely.

② After waiting for 5 minutes, rinse with tap water thoroughly.

③ Dry equipment.

*2) Sodium hypochlorite solution is corrosive to metals. If the material of the equipment can tolerate sodium hypochlorite solution, such as plastic, then it is possible to soak.

【 Micropipette 】

① Wipe entire surfaces of micropipettes gently with 2% sodium hypochlorite solution.

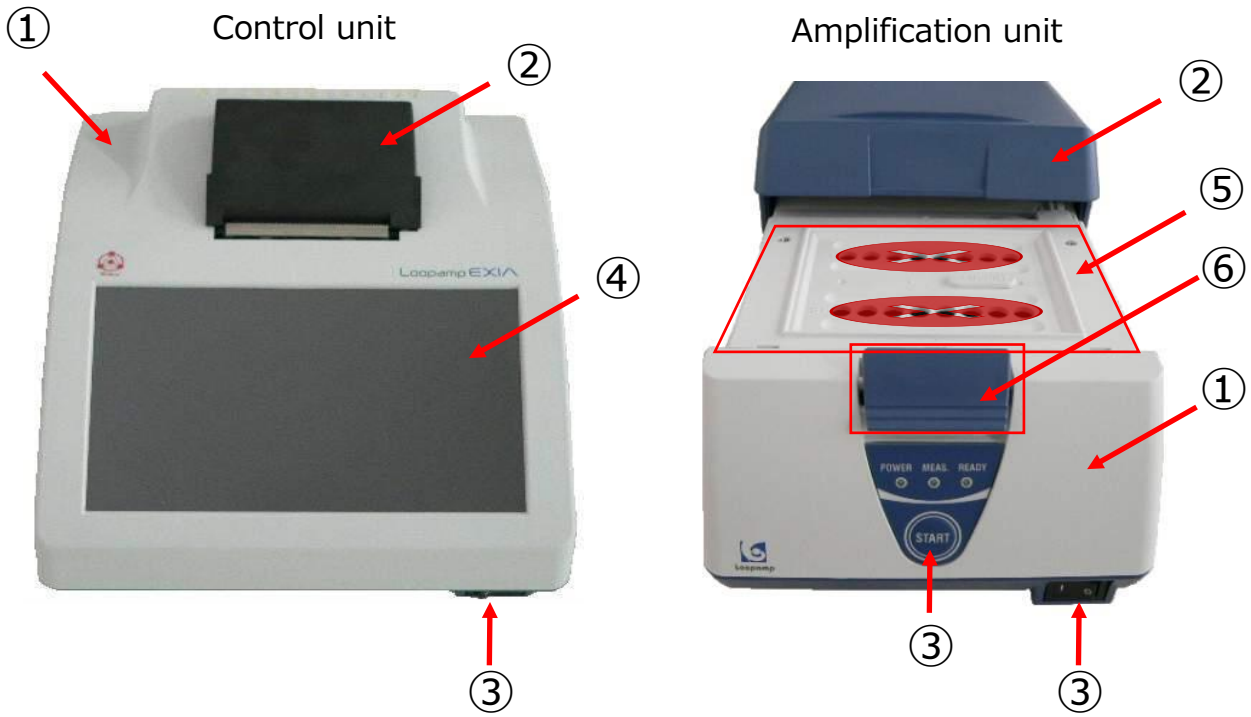
NOTE : Do not disassemble micropipettes and wipe inside to prevent corrosion.

② After leave it for 5 minutes, wipe off water with a paper towel.

③ Wipe entire surfaces of micropipettes carefully with a wet paper towel (tap water is also acceptable).

Areas to be cleaned when contamination is suspected

【 Real-Time Turbidimeter: LA-500 】



[Both units]

① Exterior

② Covers (a printer cover and a bonnet cover)
* ①, ②: clean areas touched by hands mainly.

③ Switches

[Control unit]

④ A touch panel

[Amplification unit]

⑤ Sub-cover (after opening a bonnet cover)

NOTE : Do not clean the inside of each hole (where reaction tubes are placed) since there is the light source.

⑥ Locking lever

【 How to wipe 】

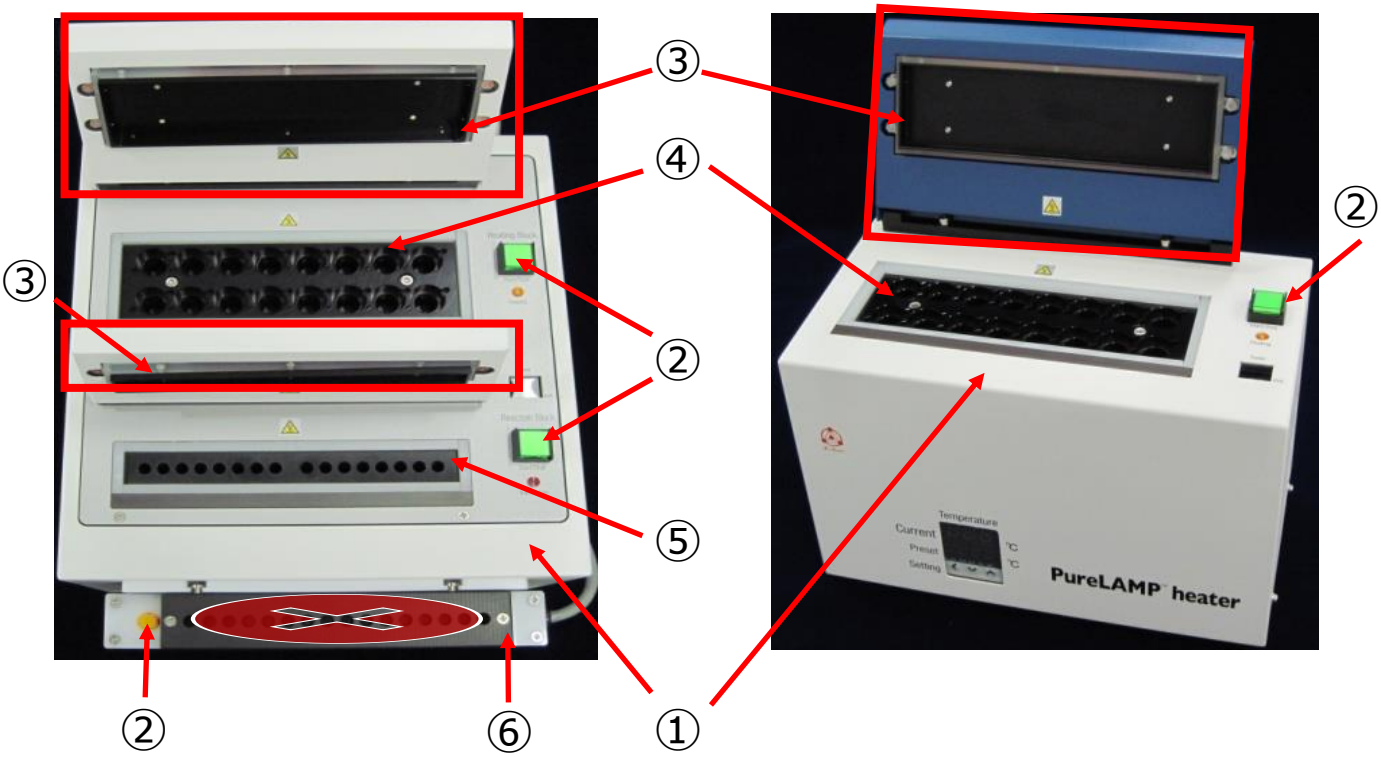
・Wipe gently with 2% sodium hypochlorite solution using a paper towel.

・Wait for 5 minutes, and wipe off water with a paper towel.

・Wipe gently enter surfaces of the instrument by a paper towel which is soaked by water. (use distilled water as much as possible)

【 LF-160 】

【 PureLAMP heater 】



[Both instrument]

① Exterior

※ Clean areas touched by hands mainly.

② Each switch (The power switch is the backside of the instrument)

③ Covers (all sides and inside of each bonnet cover)

④ Heating Block（Holes)

[LF-160]

⑤ Reaction Block

⑥ Fluorescence visual check unit

NOTE：Do not clean holes.

【 How to wipe 】

- Wipe gently with 2% sodium hypochlorite solution using a paper towel.
- Wait for 5 minutes, and wipe off water with a paper towel.
- Wipe gently enter surfaces of the instrument by a paper towel which is soaked by water. (use distilled water as much as possible)

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