



RNA/DNA Amplification Reagent D

This product is a reagent for research purpose. Do not use this product for making or supporting a diagnosis. Read this explanatory leaflet carefully before use.

【Introduction】

The LAMP (Loop-mediated Isothermal Amplification) method is a gene amplification technique characterized by (1) isothermal gene amplification reaction^{1),2)}, (2) high specificity due to the use of 4 primers recognizing 6 regions, (3) high amplification efficiency resulting in amplification in a short time, (4) a large amount of amplification product facilitating simple detection^{3),4)}.

This product is designed for amplifying and detecting the target gene sequence with the LAMP method by combining with an originally designed LAMP primer or a separately provided primer set product. This product is a dry formulated nucleic acid (RNA/DNA) amplification reagent and allows the amplification of the target gene only by dissolving the amplification reagent in a primer solution and a sample solution containing template RNA or DNA and keeping the reagent at a constant temperature. Furthermore, this product allows visual detection (hereinafter referred to as fluorescent/visual detection) using an ultraviolet irradiating device.

【Contents】

	For 96 tests
Dried RNA/DNA Amplification Reagent	48 tubes × 2

【Measurement Principle】

The LAMP method is an isothermal gene amplification technique using 4 primers and a DNA polymerase with strand displacement activity for reaction. Of the 4 primers, 2 inner primers recognize 2 different regions in the target gene sequence on their 3' and 5' sides. The sequence on the 5' side is set so that it will be annealed in the complementary strand region synthesized by the elongation reaction from the 3' side.

This amplification reaction proceeds by alternating the self-elongation from the stem loop structure generated from the inner primer and strand displacement synthesis from the inner primer annealed to the loop part. This process of the LAMP method allows isothermal amplification using only 1 enzyme. Even when RNA is used as a template, the process from the synthesis of cDNA to amplification of nucleic acid (RT-LAMP reaction) can be performed as a single step because reverse transcriptase is included in the amplification reagent. Calcein included in this reagent allows easy fluorescent/visual detection of the presence/absence of the LAMP reaction⁴⁾.

For more information on the reaction principle, please visit the Eiken GENOME SITE (URL; <http://loopamp.eiken.co.jp/e/>).

【Instructions for use】

1. Essential apparatus, equipment, and reagents, etc.

(Not included in the product. Prepare them separately)

- 1) Sterilized tube for preparing primer mix (0.5 mL or 1.5 mL)
- 2) Micropipette
- 3) Pipette tip with filter (DNase, RNase-free)
- 4) Aluminum rack for cooling reaction tubes
- 5) Ice (crushed ice) and ice box
- 6) Simple microvolume centrifuge
- 7) 8-microtube simple centrifuge
- 8) Vortex mixer
- 9) Loopamp Control Set RNA/DNA (separately provided by Eiken Chemical) ※1
Primer mix H1P (PM H1P)
Positive control H1P (PC H1P)
Negative control (NC)

A. Real-time turbidity detection
Real-time Turbidimeter (designed for LAMP method) ※2

- B. Fluorescent/visual detection
- 1) Real-time Turbidimeter (designed for LAMP method) ※2 or incubator (with a temperature precision of within ±0.5°C; with hot bonnet)
 - 2) Heat block (for enzyme deactivation) ※2
 - 3) Ultraviolet irradiating equipment (wavelength: 240 to 260 nm and 350 to 370 nm) ※2
 - 4) Wide eyeglasses or shield

※1 : Control set to be used with this product

※2 : For more information on compatible equipment, deactivation (or inactivation), and ultraviolet irradiation conditions, please visit the Eiken GENOME SITE (URL; <http://loopamp.eiken.co.jp/e/>).

2. Primer design

Appropriate primer design is an important factor to the amplification by the LAMP method. Primer design-support software dedicated for the LAMP method, "LAMP Method Primer Design Support Software, Primer Explorer," is available for designing primers on the following website: Eiken GENOME SITE (URL; <http://loopamp.eiken.co.jp/e/>).

To select appropriate grade for purification of primer, please note that the rate of reaction is increased and reaction becomes more stable as the degree of refining for a primer is increased. Therefore, simple column refining or higher grade is recommended for synthesizing primers for primary screening. HPLC refining grade is recommended for deciding a primer or after decision for at least FIP and BIP.

3. Reagent preparation method

- 1) Take a necessary number (total number of samples and controls) of the Dried RNA/DNA Amplification Reagent. To cut a bridge of reaction tube, use scissors to avoid any impact on the dried reagent (do not tear the tube). Restore the remaining reaction tube to the original aluminum pack and seal it for storage.

- 2) Preparation of primer mix (**operation on ice**)

Prepare a necessary number of each primer for the test in a separately prepared sterilized tube for preparing primer mix according to the ratio in the following table (per test). Adjust the volume of the primer mix and sample solution so that the total volume of the LAMP reaction solution will be 25.0 μL. If a separately provided primer set is combined, follow the instruction manual of the primer set.

- For sample reaction (example)

<Reagent>	<Dose>
Distilled Water (DW)	X μL (Appropriate amount)
Primer : FIP	40 pmol
BIP	40 pmol
LF ^{※3}	20 pmol
LB ^{※3}	20 pmol
F3	5 pmol
B3	5 pmol
Total	15.0 μL/test

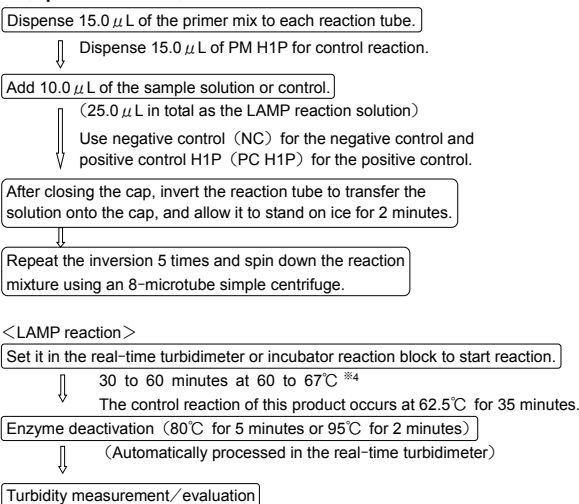
※3 : The use of Loop primer, which is not essential, reduces amplification time to approximately one third⁵⁾.

- For control reaction of this product (Loopamp control set RNA/DNA)

<Reagent>	<Dose>
Primer mix H1P (PM H1P)	15.0 μL/test

- 3) Mix by tapping or inverting the tube or using a vortex mixer for 1 second 3 times and spin down before using as the primer mix. (**Operation on ice**).

4. Operating procedure (Operation on ice)



※4 : The optimum condition for the designed primer varies and has to be individually examined.

5. Detection

- A. Real-time turbidity detection

The target gene can be detected in real time using the real-time turbidimeter (designed for the LAMP method). Refer to the package insert or operation manual, etc. for the detailed operating procedure.

B. Fluorescent/visual detection

Judgment is made by emitting ultraviolet ray to the tube from the bottom using the ultraviolet irradiation device and observing the reaction tube from the side with the eyes protected by eyeglasses, etc. Evaluate the reaction tube according to the following criteria after confirming that the positive control H1P (PC H1P) produces green fluorescence and the negative control (NC) produces no fluorescence. Take the picture of the reaction tube using a digital camera, etc. for documentation purposes, if required.

Positive: Produces green fluorescence.

Negative: Produces no fluorescence.

6. Amplification curve pattern

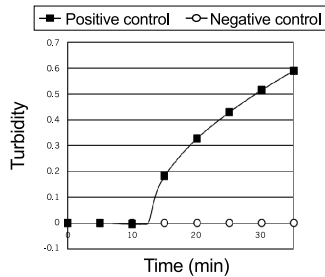


Figure 1. Control amplification curve pattern

<Precautions for measurement>

1. Because the LAMP reaction is very sensitive, any contamination with only a minute amount of the target gene or amplification product may result in an incorrect result. To avoid such contamination, the use of this product and specimen collection/nucleic acid extraction procedure should be performed in separate rooms or in different areas by partitioning the laboratory area. Take appropriate measures to prevent contamination including the use of clean benches, gloves, and isolation gowns, as required.
2. Avoid the contamination by microorganisms or nucleic acid degrading enzymes (such as DNase and RNase) in handling this product.
3. Completely dissolve the dry reagent. Incomplete dissolution may result in poor performance including low sensitivity. Do not leave more than 2 minutes in state of inverting reaction tubes.
4. Air bubbles may appear on the liquid level of the reaction solution after mixing the sample solution. Remove them to prevent measurement errors by spinning down the reaction mixture.
5. Never open the tube cap after reaction. Particularly, carefully remove the tube from the equipment so as not to open the cap after reaction. The contamination with amplification products not only results in an erroneous decision, but also causes the contamination of the measurement environment. Such contamination may persistently inhibit correct measurement unless it is completely eliminated.
6. Avoid handling amplification products using electrophoresis.
7. For the real-time turbidimeter or other incubator, when visual fluorescence judgment is chosen, inactivate the polymerase (for 5 minutes at 80°C, or for 2 minutes at 95°C) before judgment, otherwise false judgment will be caused.

[Precautions for handling (hazard prevention)]

1. This product is not designed as an in vitro diagnostic (IVD).
2. Carefully handle specimens as potentially infectious substances and take necessary biohazard prevention measures⁹⁾.
3. Take care not to directly gaze at the ultraviolet ray (sterilizing ray) from the lamp of the ultraviolet irradiation device for fluorescent/visual evaluation because it might cause serious damage. When it is necessary to gaze at the lamp that is on, make sure to do that through a glass plate or using wide eyeglasses or shield.
4. If the reagent accidentally enters the eyes or mouth or attaches to skin, immediately rinse it off with a large amount of water and seek medical treatment, if needed.

[Precautions]

1. Some specimens may inhibit the LAMP reaction and produce incorrect results because of their components.
2. This product should be stored as specified while avoiding **freezing** or sudden change in temperature.
3. Carefully handle reaction tubes because they are fragile.
4. Visually check reaction tubes for flaws or chaps. Flaws or chaps of reaction tubes, if any, may result in incorrect measurements or the contamination of measurement devices. The damage of a tube in the real-time turbidimeter or incubator reaction block may result in the leakage of the reaction solution, causing unrecoverable contamination or breakdown.
5. Take care not to apply excessive impact to the cap of the reaction tube (inside of the rib) because the detection reagent is kept dry and retained in it. Avoid the direct contact between the inside of the cap and hands.
6. Restore the remaining reaction tube to the original aluminum pack. Confirm that it is surely sealed and store it as specified.
7. Keep the positive control H1P (PC H1P) and potentially positive specimens away from other reagents.
8. Perform gene test with this product only under the supervision of experts with the knowledge and experience of gene test because the lack of knowledge or experience may result in incorrect judgment of the test result.

9. Eiken Chemical Co., Ltd. does not bear any responsibility for false judgment or any consequential damage derived from the false judgment caused by non-capability problems such as operation error.
10. Use this product within the expiration date.
11. Do not recycle the containers or accessories of this product or use them for other purposes.
12. Do not expose reaction tubes and tubes for preparing primer mix to UV light. A change in color or degeneration caused by ultraviolet lamp sometimes results in misjudgment.

[Precautions for disposal]

1. Appropriately dispose of tubes after reaction with the cap closed by putting them in double plastic bags that can be incinerated or sealed. To prevent dispersion of amplification products, **do not autoclave tubes before disposal**.
2. Reaction tubes are mainly made of polypropylene (PP). The tray for reaction tubes is mainly made of PET. The aluminum pack is mainly made of aluminum. The case is mainly made of paper.
3. Dispose of this product, containers, and materials before or after use on the responsibility of the laboratories in compliance with applicable laws on waste disposal and cleaning and water pollution prevention law.

[Storage method, shelf life, packaging unit, and product code]

Product name	Storage method	Shelf life	Package unit	Product code
Loopamp™ RNA/DNA Amplification Reagent D	1-30°C	1 year	For 96 tests	LMP247

[References]

- 1) Notomi T. et al., : Nucleic Acids Research, **28** (12) :e63, 2000.
- 2) Nagamine K. et al., : Clin. Chem, **47** (9) :1742-1743, 2001.
- 3) Mori Y. et al., : Biochem. Biophys. Res. Commun, **289** (1) :150-154, 2001.
- 4) Tomita N. et al., : Nat Protoc, **3** (5) :877-882, 2008.
- 5) Nagamine K. et al., : Molecular and Cellular Probes, **16** (3) :223-229, 2002.
- 6) The guideline for the bio-safety and bio-hazard (by the Japanese Society for Bacteriology) : Japanese Journal of Bacteriology, **54** (3) :667-715, 1999.

Manufacturer



EIKEN CHEMICAL CO., LTD.

143 Nogi, Nogi-machi, Shimotsuga-gun, Tochigi 329-0114, Japan

Date of Revision : April 2015 (Ver.1)