For research use only

Loopamp™
LAMP (Loop-mediated Isothermal Amplification) method

Control Set RNA/DNA

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 a control reaction kit to be combined with Loopamp RNA/DNA Amplification Reagent D（separately provided by Eiken Chemical）.

【Contents】

	For 12 tests
1．Primer mix H1P（PM H1P）	0.72 mL × 1
2．Positive control H1P（PC H1P）	0.16 mL × 1
3．Negative control（NC）	0.16 mL × 1

【Instructions for use】

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

Refer to the instruction manual of Loopamp RNA/DNA Amplification Reagent D.

2．Reagent preparation method（follow the procedure of Loopamp RNA/DNA Amplification Reagent D for details）.

1）Take a necessary number（total number of samples and controls）of the Dried RNA/DNA Amplification Reagent. Immediately return the remaining reagent to the original aluminum pack and seal it.

○ For control reaction（this product）

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

2）Mix the reagent（PM H1P）by inverting, tapping or using a vortex mixer for 1 second 3 times and spin down before using as the primer mix **（Operation on ice）.**

3．Operating procedure
（Operation on ice）

Dispense 15.0 μL of PM H1P to each reaction tube.
⇩
Add 10.0 μL of the control to each tube.
（25.0 μL in total as the LAMP reaction solution）
Use negative control（NC）for the negative control and positive control H1P（PC H1P）for positive control.
After closing the cap, invert the reaction tube to transfer the solution onto the cap, and allow it to stand on ice for 2 minutes.
⇩
Repeat the inversion 5 times and spin down the reaction mixture using an 8-microtube simple centrifuge.
〈LAMP reaction〉
Set it in the real-time turbidimeter or incubator reaction block to start reaction.
⇩ The control reaction of this product occurs at 62.5℃ for 35 minutes.
Enzyme deactivation（80℃ for 5 minutes or 95℃ for 2 minutes）
⇩（will be automatically processed in the real-time turbidimeter）
Turbidity measurement/evaluation

4．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 reaction 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（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.

5．Amplification curve pattern

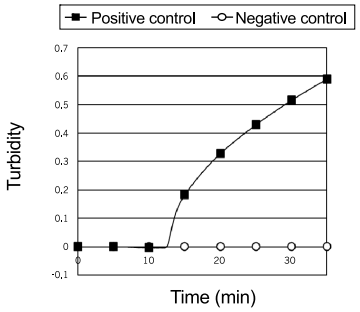

Figure 1. Control amplification curve pattern

＜Precautions for measurement＞

1．Because the LAMP reaction is very sensitive, any contamination with extremely minimum 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.

【Precautions for handling（hazard prevention）】

1．This product is not designed as an in vitro diagnostic（IVD）.
2．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 is dangerous. 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.
3．A minute amount of sodium azide is contained as a preservative in the primer mix H1P（PM H1P）, positive control H1P（PC H1P）, and negative control（NC）. Take care to prevent sodium azide from entering the eyes or mouth or attaching to skin because it is toxic.
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．This product should be stored as specified while avoiding **freezing** or sudden change in temperature.
2．Keep the positive control H1P（PC H1P）away from other reagents.
3．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.
4．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.
5．Use this product within the expiration date.
6．Do not recycle the containers or accessories of this product or use them for other purposes.
7．Do not use it in combination other regents with the Loopamp RNA/DNA Amplification Reagent D.

【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．The constituent reagent tube is mainly made of polypropylene（PP）. The kit 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™ Control Set RNA/DNA	2-8℃	1 year	For 12 tests	LMP248

【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.

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