Read this instruction carefully before use

For research use only

Rev. January 2008 (Ver.3)

Loopamp®
LAMP (Loop-mediated Isothermal Amplification) method

Primer Set for West Nile Virus

【Characteristics】

LAMP (Loop-mediated Isothermal Amplification) method is a novel gene amplification method capturing the following characteristics : ① Only one enzyme is required and the amplification reaction proceeds under isothermal condition[1), 2)]., ② It has extremely high specificity because of the use of 4 primers recognizing 6 distinct regions on the target., ③ It has high amplification efficiency and enables amplification within a shorter time., ④ It produces tremendous amount of amplified products which makes simple detection possible[3), 4), 5), 6)].

This reagent consists of the primer set for the detection of the genomic RNA encoding envelope of West Nile Virus (WNV)[7)], which is specifically designed for Loopamp RNA Amplification Kit (RT-LAMP).

【Contents of the kit】

	48 tests
(1) Primer Mix. WNV (PM WNV) *1	0.12 mL × 1 tube
(2) Positive Control WNV (PC WNV) *1	60 μL × 1 tube

*1 : The notation on each reagent tube is shown in ( ).

【How to use】

1. Materials required but not provided

Refer to the package insert for Loopamp RNA Amplification Kit (RT-LAMP).

2. Preparation of the specimen

Carry out the extraction of RNA from the specimen using a commercially available virus genome isolation reagent (for example, QIAamp Viral RNA Mini Kit, Qiagen). When handling the sample, always abide by the biohazard counter measures[8)].

3. Reagents preparation

1) Take out the reagents stored at −20℃, and thaw them at room temperature. Once the reagents are thawed, keep them on ice.

2) Preparation of master mix. (Operate on ice).
Referring to the table below, dispense the necessary amount of Primer Mix. WNV (PM WNV) of this kit, and 2 × Reaction Mix. (RM), Enzyme Mix. (EM) and Distilled Water (DW) of Loopamp RNA Amplification Kit (RT-LAMP), into sterilized tube for master mix.

<Reagents>	<Amount : 1 test>	<Amount : 10 tests>
2 × Reaction Mix. (RM)	12.5 μL	125 μL
Primer Mix. WNV (PM WNV)	2.5 μL	25 μL
Enzyme Mix. (EM)	1.0 μL	10 μL
Distilled Water (DW)	4.0 μL	40 μL
Total	20.0 μL	200 μL

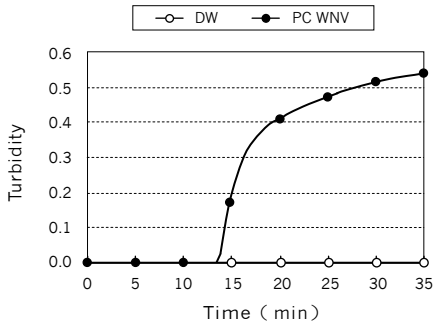
4. Operation procedures (Follow the instruction of Loopamp RNA Amplification Kit (RT-LAMP)).

1) Mixing master mix. solution and the sample solution (Operate on ice).
(1) Dispense 20 μL of the master mix. into each Loopamp Reaction Tube.
(2) Add 5 μL of sample RNA, Positive Control WNV (PC WNV) or Negative Control (DW) into the master mix., and the volume of the solution should be 25 μL in total. Mix the solution well by pipetting or tapping the tube and then spin down. Be careful not to cause air-bubbles when mixing.

2) Amplification and detection
(1) Incubate at 63℃ for 35 minutes.
(2) After the amplification reaction, use a heat block to inactivate the polymerase and terminate the reaction (5 minutes at 80℃ or 2 minutes at 95℃).

☆ If other final judgment procedures such as fluorescence visual detection or electrophoresis is to be taken, be sure that the enzyme is inactivated.
For information on fluorescence visual detection, refer to the package insert for Loopamp Fluorescent Detection Reagent.

☆ For examples of detection cases using this reagent, refer to the products introduction page in Eiken GENOME SITE (URL; http://loopamp.eiken.co.jp/e/)



The amplification curve of positive control WNV RNA*2
(monitored by Loopamp Realtime Turbidimeter)

*2 : There is no correlation between the initial template number and the turbidity increment time.

【Caution for Handling】

1. This reagent is designed for research use only.
2. If the operator does not have the experience or knowledge in the field of nucleic acid testing, there's a possibility of false judgment. Therefore, make sure that the reagent is used under the supervision of the experienced and knowledgeable technicians.
3. 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...
4. Use the reagent before the expiration date, which is labeled on the outer box (Exp.Date).
5. The reagent tube is made of polypropylene and the main material for kit case is paper. The institution disposing the reagent tube and case should bear the responsibility and abide by the clinical waste disposal regulations, water pollution prevention law, and any other regulation related.

【Unit, Storage, Expiration, Code No.】

Product Name	Unit	Storage	Expiration	Code No.
Loopamp® Primer Set for West Nile Virus	48 tests	−20℃	1 year	PM0001

【References】

1) Notomi T. *et al.* : Nucleic Acids Research **28**, No.**12**, e63 (2000)
2) Nagamine K. *et al.* : Clin. Chem. **47**, No.**9**, 1742-1743 (2001)
3) Mori Y. *et al.* : Biochem. Biophys. Res. Commun. **289**, No.**1**, 150-154 (2001)
4) Tomita N. *et al.* : Abstract for The 73rd Annual Meeting of the Japanese Biochemical Society (2000)
5) Mori Y. *et al.* : Abstract for the 23rd Annual Meeting of the Molecular Biology Society of Japan (2000)
6) Tomita N. *et al.* : Abstract for the 26th Annual Meeting of the Molecular Biology Society of Japan (2003)
7) Parida M. *et al.* : Journal of Clinical Microbiology **42**, No.**1**, 257-263 (2004)
8) The guideline for the bio-safety and bio-hazard (by the Japanese Society for Bacteriology) : Japanese Journal of Bacteriology **54**, No.**3**, 667-715 (1999)

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