Handbook

FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit (Reverse-Transcription Loop-Mediated Isothermal Amplification)

120 reactions (60 tests/kit)

For *in vitro* Diagnostic (IVD) Use

ZENOSTIC

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INSTRUCTIONS FOR USE: FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit

1. CATALOG NUMBER

ZE-P14

2. PACKAGE SPECIFICATION

120 reactions (60 tests/kit)

3. INTENDED USE/ INDICATIONS FOR USE

FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit is a qualitative *in vitro* diagnostic test for the detection of nucleic acids from SARS-CoV-2 in nasopharyngeal and oropharyngeal (throat) swab from individuals who are suspected of COVID-19.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during infection. Positive results are indicative of SARS-CoV-2 RNA.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

4. PRODUCT OVERVIEW/ TEST PRINCIPLES

This product is based on the nucleic acid amplification technique, Reverse-Transcription Loop-Mediated Isothermal Amplification (RT-LAMP). Only one enzyme is required and amplification reaction proceeds under isothermal conditions; it has an extremely high specificity because of the use of four primers recognizing six distinct regions on the target; it has a high amplification efficiency and enables amplification within a short time; and it produces tremendous amount of amplified product which makes simple visual detection possible.

The primers provided with this product have been designed in the N gene of SARS-CoV-2 RNA and human RNase P gene as an internal control. This region has been confirmed well-conserved in the selected nucleic acid sequences of SARS-CoV-2 by alignment analysis.

RNA from clinical samples, nasopharyngeal, oropharyngeal (throat) swab samples are extracted using MagDEA® Dx reagents (Precision System Science, Japan), GENTi™ Viral DNA/RNA Extraction Kit (GeneAll, Korea), QIAamp Viral RNA Mini Kit (Qiagen,

Germany), QIAamp MinElute Virus Spin Kits (Qiagen, Germany), Sera-Xtracta Virus/Pathogen Kit (Cytiva, USA).

The detection of amplified products is based on the color change of the indicator dye (phenol red) from pink to yellow of the product, the assay relies on the pH change of the RT-LAMP solution during the amplification phase, The color change was visualized by the naked eye.

5. PRODUCT COMPONENTS

No.	Components	Volume	Main Ingredients
1	Color LAMP-TTR (ZE-P15)	1,200 µL/tube	Reverse transcriptase, BST DNA polymerase, $MgSO_4$, Uracil-DNA glycosylase (UDG)
2	N primers mix-TTR (ZE-P16)	300 µL/tube	Inner primer (FIP and BIP), outer primer (F3 and B3), and loop primer (LF and LB)
3	RNase P primers mix-TTR (ZE-P17)	300 µL/tube	Inner primer (FIP and BIP), outer primer (F3 and B3), and loop primer (LF and LB)
4	Positive control-TTR (ZE-P18)	60 µL/tube	In vitro transcriptional RNA for N gene and human RNase P gene (internal control)
5	Negative control-TTR (ZE-P19)	60 µL/tube	RNase-Free Water

6. REAGENT STABILITY AND TRANSPORTATION

6.1 Storage conditions

The kit (in small box) should be stored at -20 ±5°C and should be transported in a sealed foam box with ice packs . The sample storage reagent should be transported at 2-8°C or below. The performance of the kit during simulated shipping condition was stable up to 24 h of transportation time. The FastProof™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit should be stored in the original packaging and is stable for up 6 months once stored at -20°C. Repeated thawing and freezing should be kept to a minimum

and should not exceed 6 freeze-thaw cycles. Always check the expiration date prior to use. Do not use expired reagents.

6.2 In Use Stability

FastProof™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit should be stored in the original packaging and is stable for up to six months after manufacturer's date and stored at -20°C. The kit should not be used past the "use by" date as indicated on the pack label and individual tube labels. When in use the kit components should be returned to the freezer promptly after use to minimize the time at room temperature. Repeated thawing and freezing should be kept to a minimum and should not exceed 6 freeze-thaw cycles. Components may be aliquoted into smaller volumes, if required.

7. MATERIAL REQUIRED BUT NOT SUPPLIED

Reagents for RNA extraction:

MagDEA® Dx reagents (Precision System Science, Japan), GENTi™ Viral DNA/RNA Extraction Kit (GeneAll, Korea), QIAamp Viral RNA Mini Kit (Qiagen, Germany), QIAamp MinElute Virus Spin Kits (Qiagen, Germany), Sera-Xtracta Virus/Pathogen Kit (Cytiva, USA).

Consumables not supplied:

- 0.2-mL PCR tubes
- 1.5 – 2.0 mL microcentrifuge tube
- Automatic Pipettes (10, 200, and 1000 µL)
- Filtered Tips (10, 200 and 1000 µL)
- Mini-centrifuge Machine
- Thermal Cycler Machine or Heat Block
- Cooling racks or equivalent
- Ethanol
- RNase away reagent
- Disposable powder-free gloves

8. WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- This product is designed only for clinical diagnosis of SARS-CoV-2 from clinical samples of human origin. Do not use for other purposes.
- When using this product, always follow this Instructions for Use.
- Do not use any expired reagent.
- Performance of the kit is dependent on operator proficiency and adherence to procedural directions. Testing should be performed by properly trained personnel.
- Read the instruction manual of equipment involved incubator before use.
- Clinical samples pose a potential risk for infection. Take all necessary preventive measures to avoid biohazard.
- In case of accidental contact of any reagent with eyes, mouth, or skin, immediately rinse the affected site with plenty of water and, if necessary, seek medical advice.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- All contents in this package are prepared and validated for the intended testing purpose. Replacement or modification of any of the package contents will affect the testing performance of the kit and is in violation of the product Emergency Use Authorization. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kit lots. Prior to begin each assay, each component must be thoroughly thawed and briefly centrifuged. Avoid repeated freeze-thaw cycles.
- All pipette tips and centrifuge tubes in the assay should be sterile and DNase/RNase-free. To prevent contamination, filtered pipette tips are required and should be replaced after the addition of each reagent or sample.

9. CONTROLS MATERIALS

Positive control-TTR: A positive template control is used to monitor whether the RT-LAMP process works properly and is used in each detection run.

Negative control-TTR: A no template or negative control is used to monitor whether there is contamination for the RT-LAMP process and is used in each detection run.

An internal control for RNase P gene is used to monitor the sample collection, handling and RT-LAMP process and is used in each sample amplification.

10. LABORATORY PROCEDURE

Preparation of reagents

10.1 Thawing of solutions. Take the Color LAMP-TTR, Primers mix-TTR, positive control-TTR, and negative control-TTR (Rnase-free water) out form -20°C. Thaw all tubes at room temperature and place all tubes on cold rack at 4°C or on ice.

10.2 Preparation of RT-LAMP supermix for LAMP reactions:

Mix 10 µL the Color LAMP-TTR (ZE-P15) with 5 µL of Primers mix-TTR. Pipette up and down gently several time to mix the solution.

(Carry out in a biosafety cabinet, a laminar flow cabinet, or a clean closed-system cabinet such as a PCR cabinet workstation)

10.3 Aliquot 15 µL the RT-LAMP supermix into each of the PCR tubes.

10.4 Add 5 µL of extracted RNA to one of the reaction PCR tubes in the following order: Negative control-TTR, clinical specimen(s), and Positive control-TTR. Cover each well, spin down the reaction PCR tube. For 5 seconds.

*This step of adding Rnase-free water into the supermix (negative control) should be performed prior to adding RNA extracted from the tested samples and the RNA positive control.

Note: To prevent cross contamination, finish aliquoting the RT-LAMP supermix into the reaction tubes and preparing the negative control-TTR prior to touching the RNA templates for testing. Also, the work area for the preparation of the RT-LAMP supermix (always clean) and the work area for all activities involved with the tested RNA should be separated.

Table 1: Supermix preparation

	N gene			RNase P gene		
	Negative	Positive	Tested sample	Negative	Positive	Tested sample
Color LAMP-TTR	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL
N primers mix-TTR	5 µL	5 µL	5 µL	-	-	-
RNase P primers mix-TTR (internal control)	-	-	-	5 µL	5 µL	5 µL
Positive control-TTR	-	5 µL	-	-	5 µL	-
Negative control-TTR	5 µL	-	-	5 µL	-	-
Extracted RNA	-	-	5 µL	-	-	5 µL

10.5 Running the RT-LAMP Reaction, Incubate the reaction PCR tubes at 65°C for 30 minutes in a thermal cycler or heating block.

11. INTERPRETATION OF RESULTS

- If the reaction solution turns yellow or orange, interprets as positive result or detected.
- If the reaction solution remains pink, interprets as negative result or not detected.

Table 2: Interpretation of result

N	RNase P (Internal control)	Result
+	+	SARS-CoV-2 Positive
-	+	SARS-CoV-2 Negative
+/-	-	Invalid/ Re-test

12. LIMITATIONS

- FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit should be following from guidance and procedure.
- The performance of the FastProof™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit was established using nasopharyngeal (NP) and oropharyngeal (throat) swabs are also considered acceptable specimen types for use with the kit.

- FastProof™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit is not a gold standard method. Once SARS-CoV-2 RNA were detected by FastProof™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit, the sample must be confirmed by qRT-PCR assay (gold standard method).
- Do not eliminate feasibility of negative result, because incubation period after infection might not be enough for SARS-CoV-2 RNA detection in the early stage of infection.

13. TROUBLESHOOTING

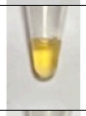
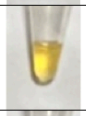
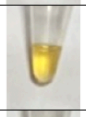
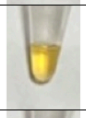
Problem	Solution
Precipitation of the master mix after repeated freeze/ thaw cycles.	- Vortex or invert the tubes several time until the precipitates disappear.
Negative control turns yellow (False positive).	- Clean your working space and equipment. - Change RNase free water. - Check the temperature control system of the thermal cycler machine. - Do not let the master mix and the reaction expose to air for too long by leaving the lid open during preparation of the master mix and the reaction. - Always keep the master mix and the reaction at 4°C.

14. PERFORMANCE EVALUATION

Limit of Detection (LoD) - Analytical Sensitivity:

The limit of detection (LoD) is defined as the lowest concentration of analyte that could be reliably detected at least 95% of the time. LoD was assessed by testing seven contrivance levels (dilutions) of SARS-CoV-2 RNA. The LoD of FastProof™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit is 1,526.76 copies/mL (***Table 3***)

Table 3 *Analytical sensitivity of FastProof™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit*

RNA concentration			Ct value (AVG ± SD)			RT-LAMP	
copies/µL	copies/rxn	copies/mL	ORF1ab	N	IC	%Positive	Color
NTC	NTC	NTC	ND	ND	ND	0	
0.14	0.71	61.07	39.89±4.81	35.91±1.37	ND	0	
0.71	3.56	305.35	38.62±2.13	34.42±0.42	ND	16.7	
3.56	17.81	1,526.76	34.85±0.93	32.09±0.61	ND	100	
4.45	22.26	1,908.44	34.52±0.50	31.92±0.60	ND	100	
8.90	44.52	3,816.89	33.80±0.36	31.40±0.28	ND	100	
17.81	89.05	7,633.78	32.47±0.10	30.00±0.10	ND	100	
89.05	445.24	38,168.88	30.37±0.11	27.84±0.07	ND	100	

Inclusivity (analytical sensitivity)

To ensure the COVID-19 primers to detect SARS-CoV-2 genomes, Primerdesign's Bioinformaticians review daily the SARS-CoV-2 sequence submission on the GISAID EpiCoV database. As of 30 of March 2021, in silico analysis confirms the COVID-19 assay primers still show 100% detection several SARS-CoV-2 sequences published on the GISAID EpiCoV database.

Cross-reactivity (Analytical Specificity):

To evaluate the specificity toward SARS-CoV-2, we tested the developed FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit (Reverse-Transcription Loop-Mediated Isothermal Amplification) assay with RNA samples of other respiratory viruses including influenza A viruses (subtypes H1 and H3), influenza B virus, RSV-A/B, hMPV, HCoV-HKU1, MERS-CoV, HCoV-OC43, HCoV-299E, and HCoV-NL63. Although we did not perform specificity tests against SARS-CoV due to lack of clinical samples, we confirmed that the assay did not cross-react with 6 common human respiratory viruses as well as five other

human coronaviruses namely MERS-CoV, HKU-1, OC43, 229E and NL63 ***(Table 4)***. These data indicate that the FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit is as specific as qRT-PCR in detecting SARS-CoV-2.

Table 4: Cross-Reactivity of FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit (Reverse-Transcription Loop-Mediated Isothermal Amplification)

Virus	Sample type	RT-LAMP (+/-)	Ratio of positive replicates (RPR)
Influenza A/H1 virus	RA202001	-	0/3
Influenza A/H3 virus	RA202002	-	0/3
Influenza B virus	RA202003	-	0/3
Influenza B virus	RA202004	-	0/3
Respiratory syncytial virus A	RA202005	-	0/3
Respiratory syncytial virus B	RA202006	-	0/3
Human metapneumovirus	RA202007	-	0/3
Human metapneumovirus	RA202008	-	0/3
Coronavirus HKU1	RA202009	-	0/3
Coronavirus OC43	RA202010	-	0/3
Coronavirus 229E	RA202011	-	0/3
Coronavirus NL63	RA202012	-	0/3
MERS-CoV	RA202013	-	0/3
SARS-CoV-2	RA202014	+	3/3

Clinical evaluation

The clinical performance of FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit (Reverse-Transcription Loop-Mediated Isothermal Amplification) was established using 224 nasopharyngeal swab specimens collected from patients who were suspected of COVID-19. The comparator method was the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) from Sansure Biotech. The FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit (Reverse-Transcription Loop-Mediated Isothermal Amplification) and Sansure Biotech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit comparator assay method was the QIAamp Viral RNA Mini Kit (Qiagen, Germany). Sansure Biotech assay was run on CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). The results are summarized in ***Table 5*** and demonstrated a

positive productive value (PPV) 98.81% and negative productive value (NPV) of 97.86%.

Table 5: Clinical evaluation between FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit (Reverse-Transcription Loop-Mediated Isothermal Amplification) and Sansure Biotech Novel Coronavirus (2019-nCoV) Comparator Method

Test		Sansure Biotech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit		Total
		qRT-PCR Positive	qRT-PCR Negative	
FastProof ™ 30 min-TTR SARS-CoV-2 RT-LAMP Kit	RT-LAMP Positive	83	1	84
	RT-LAMP Negative	3	137	140
	Total	86	138	224
Sensitivity (95% CI)				96.51% (90.14%, 99.27%)
Specificity (95% CI)				99.28% (96.03%, 99.98%)
Positive predictive value (95% CI)				98.81% (92.17%, 99.83%)
Negative predictive value (95% CI)				97.86% (93.76%, 99.28%)

15. STORAGE CONDITION

The kit should be stored at -20 ±5°C.

16. SHELF LIFE

6 months

17. SYMBOLS

Symbols	Meanings
IVD	In vitro diagnostic medical device
	Date of manufacture
	Expiratory date (last date of month)

18. CONTACT INFORMATION AND PRODUCT SUPPORT

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