TOOLSee Saliva Colorimetric RT-LAMP kit

Quick and Visible detection for amplification from saliva sample

Contents

Introduction

The TOOLSee Saliva Colorimetric RT-LAMP kit offers simple, time-saving, and affordable method including saliva extraction and direct RT-LAMP reaction to detect target gene in form of RNA from saliva via reverse transcription loop-mediated isothermal amplification (RT-LAMP). Saliva is an excellent and non-invasive source of RNA for rapid testing. The saliva extraction is suitable and stable for nucleic acids analysis by simple mix and heating. The extraction is able to be subjected to following analysis by RT-LAMP reactions contains optimized indicator to the RT-LAMP reaction master mix without inhibiting amplification efficiency. The reducing concentration of Mg+2 in RT-LAMP reaction leading to a color change from violet to sky blue. This Saliva Colorimetric RT-LAMP kit can be used for saliva extracted sample and applies to detection of virus and micro pathogens. The end amplicon of TOOLSee Colorimetric RT-LAMP kit can be verified by agarose gel electrophoresis.

Important Notes

- Before the experiment, please wipe the bench surface with fresh 5-10% bleach, and then clean the bench surface with 75% alcohol to reduce the contamination of nucleic acid residues.

Kit Contents

Contents	ST-SL01 (100 rxn)
Quick saliva extraction reagent	20 mL
2X RT-LAMP master mix	1,000µL
10X colorimetric indicator	200 µL

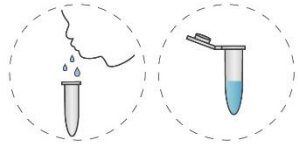
Storage

- 2X RT-LAMP Master Mix should be stored at -20°C.
- Quick saliva extraction reagent and 10X colorimetric indicator should be stored at room temperature.

Materials needed but not supplied with the product

- Proteinase K (20mg/vial) (TOOLS, Cat# TTG-PKP01)

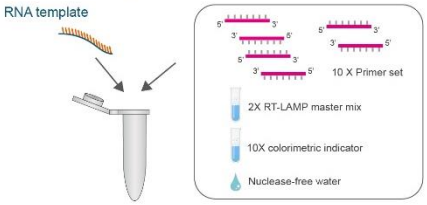
Work Flow



Collect saliva sample and prepare the extraction reagent

Homogenize saliva sample with the extraction reagent

Heat at 95°C for 15 mins



Prepare the RT-LAMP reaction

Incubate at 63°C for 30-60 min

Positive reaction

Protocol

1. Prepare the extraction reagent in the proportion of 20 µL Proteinase K (20 mg/mL) (TOOLS, Cat# TTG-PKP01) and 200 µL TOOLS Quick Saliva Extraction Reagent. The volume could be scaled up according to the number of samples.

2. Homogenize the saliva sample with the reagent prepared in step 1 at 1:1 ratio. Generally, add 200 µL the reagent prepared in step 1 to 200 µL saliva sample and mix thoroughly by pulse-vortexing.

3. Incubate the homogenized sample at 95 ºC for 15 minutes to permit complete lysis. During incubation, vortex the sample for every 5 minutes.

4. Place the extracted sample on the ice, ready for RT-LAMP.

5. Prepare the RT-LAMP reactions by the below table

Component	18 µL/rxn	Final Conc.
2X RT-LAMP Master Mix	10 µL	1X
10X colorimetric indicator	2 µL	1X
40 µM FIP/BIP primer	1 µL	1.6 µM
10 µM F3/B3 primer	1 µL	0.4 µM
20 µM Loop F/Loop B primer	1 µL	0.8 µM
Nuclease-Free water	3 µL	-

6. Add 2 µL the saliva extracted sample to the RT-LAMP reaction tube in a total volume of 20 µL. Mix the reaction thoroughly by brief vortex and spin down.

7. Incubate at 63°C for 30-60 min.

8. After RT-LAMP reactions complete, inactivate the enzyme by heating at 80°C for 10 min.

9. Take reaction tubes from incubation and observe the color change by eyes. Positive reactions will result in sky bule, while negative controls should remain violet.

Troubleshooting

Problem	Cause	Solution
False-positive result	Contamination	Prepare fresh reagent, clean equip and working area with 10% bleach solution, wear glove and mask.
False-negative result	RNA degradation	Prepare fresh RNA template, place on ice during performing experiment.
	Incorrect primer concentration	Use recommended primer concentration.
	Improperly mix	Mix with pipetting.
	Insufficient template	Increase RNA template.
No amplification	Insufficient reaction time	Increase reaction time (up to 60 minutes).
	Improperly mix	Mix with pipetting.
	Affected by component of sample	Run reaction with diluted sample.