TOOLSee Colorimetric RT-LAMP kit

Visible and one-step detection for amplification from RNA

Contents

Introduction

TOOLSee Colorimetric RT-LAMP kit is a dual enzyme system including an optical indicator which offers a simple and one step solution to detect target gene in form of RNA via reverse transcription loop-mediated isothermal amplification (RT-LAMP) simply in a fixed temperature within 30 to 45 minutes. This kit contains optimized metal intercalating indicator of which color changes from violet to sky blue reflecting the reducing concentration of Mg^{+2} for quick and easy detection of RT-LAMP reaction master mix inhibiting amplification efficiency. This kit was validated and used to detect RNA from purified nucleic sample and compatible with crude extract samples such as saliva, which makes TOOLSee Colorimetric RT-LAMP kit ideal for the detection of virus and micro pathogens. The end amplicon of TOOLSee Colorimetric RT-LAMP kit can be also 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.

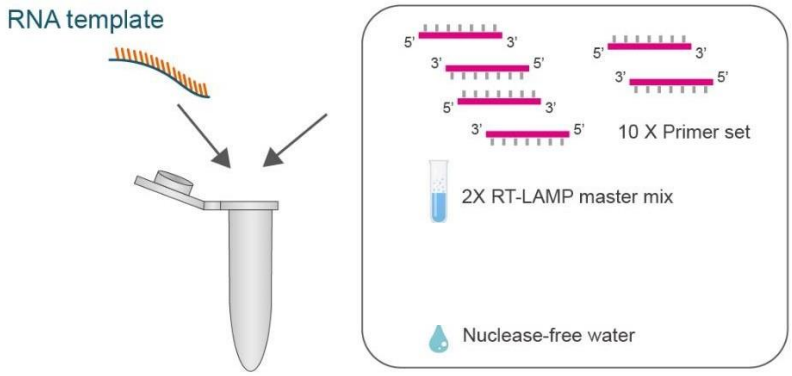
Kit Contents

Contents	ST-L02
2X RT-LAMP master mix	1,000 µL

Storage

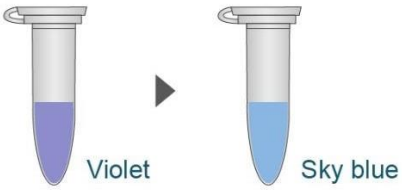
- 2X RT-LAMP Master Mix should be stored at -20°C.

Work Flow



Prepare the RT-LAMP reaction

Incubate at 65°C for 30-45 min



Positive reaction

Protocol

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

Component	25 µL/rxn	Final Conc.
2X RT-LAMP Master Mix	12.5 µL	1X
40 µM FIP/BIP primer	1 µL	1.6 µM
5 µM F3/B3 primer	1 µL	0.2 µM
20 µM Loop F/Loop B primer	1 µL	0.8 µM
RNA template	1-2 µL	variable
Nuclease-Free water	Fill to 25 µL	-
Total reaction volume	25 µL	-

2. Mix the reaction thoroughly by brief vortex and spin down.
3. Incubate at 65°C for 30-45 min.
4. After RT-LAMP reactions complete, inactivate the enzyme by heating at 80°C for 10 min.
5. 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