SuperScript™ IV RT-LAMP Master Mix

QUICK REFERENCE Pub. No. MAN0025703 Rev. A.0

invitrogen

Contents and storage

Component	Cat. No.			Storage
	A51801 (100 Rx)	A51802 (400 Rx)	A51803 (1000 Rx)	
SuperScript™ IV RT-LAMP Master Mix	1.25 mL	4 × 1.25 mL	10 × 1.25 mL	Store at –20°C Protect from light
SYTO™ Green Fluorescent Nucleic Acid Stain	30 µL	30 µL	30 µL	

Product description

The Invitrogen™ SuperScript™ IV RT-LAMP Master Mix (2X) is a ready-to-use mixture optimized for reverse transcription loop-mediated isothermal amplification (RT-LAMP) of purified viral RNA in 5–30 minutes which is compatible with real-time or end-point detection. RT-LAMP is a single-tube method of rapid and specific nucleic acid amplification under isothermal conditions (e.g., 65°C) allowing the procedure to be performed with only a block heater.

The master mix is compatible with SYTO™ 9-based real-time fluorescent RT-LAMP assays and end-point detection. Results can also be determined by visual evaluation of color change using SYBR™ Green I nucleic acid stain or agarose gel electrophoresis.

- The SuperScript™ IV RT-LAMP Master Mix (2X) includes SuperScript™ IV Reverse Transcriptase, evolved Bst DNA polymerase, optimized RT-LAMP buffer and dNTPs.
- The SuperScript™ IV Reverse Transcriptase (RT) is a proprietary MMLV mutant with superior robustness and better processivity. It provides high reproducibility and reliability, and ensures fast synthesis of the cDNA.
- The evolved Bst DNA polymerase has strong strand displacement activity, and ensures superior amplification speed.
- SYTO™ 9 Green Fluorescent Nucleic Acid Stain is supplied with the kit, making it possible to perform real-time fluorescence detection immediately upon completion of the RT reaction.

Online resources

- Visit thermofisher.com for additional information and protocols.
- For support, visit thermofisher.com/support.

Important guidelines

- Follow cleaning instructions (see **Cleaning instructions**) to minimize risk of environment-borne contamination.
- Use sterile filtered pipette tips to prevent aerosol contamination.
- It is recommended to use nuclease-free, non-stick, low-binding surface tubes for maximum performance. Learn more about preventing RNA degradation at thermofisher.com/rnase.
- Negative control reactions are recommended (e.g., no template control using target-specific primer sets) to demonstrate lack of background amplification.
- Multiple technical replicates are recommended.
- The RT-LAMP reaction mix should be kept on ice during the reaction set-up.
- Analyze RT-LAMP amplicons by end-point detection method in a workspace separate from the RT-LAMP reaction set-up area.
- Primer design is crucial for successful amplification of the RNA target.
- It is highly recommended to add Loop primers to the reaction mix for the greatest amplification speed and sensitivity.

General guidelines for primer design

For primer design and optimization, publicly available web-based tools such as PrimerExplorer (http://primerexplorer.jp/e/) can be used.

To order primers, go to thermofisher.com/primers.

Critical aspects of LAMP primer design include:

- Amplicon length <300 bp
  - Distance between F2 and B2 primers is 120–160 bp
  - Loop (distance between F1 and F2 primers) is 40–60 bp
- 45-60 % GC content, avoid single or dinucleotide base repeats and secondary structure regions.
- Ensure primer stability such that the 3'-end of F2/B2 and F3/B3, and the 5'-end of F1c/B1c has a free energy of -4 kcal/mol or less.
- Ensure similar melting temperature (Tm) across primer pairs (< 5°C difference).

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Contamination prevention

It is important to prevent environment-borne and carry-over contamination that can result in non-specific amplification. Observe the following guidelines when performing procedures involving RNA detection.

- Always wear a clean laboratory coat and gloves while performing the experiment. Wash hands prior to the work in the laboratory.
- Change gloves frequently during the experiment. Changing gloves after cleaning the laminar flow hood, and prior to adding the NTC is recommended.
- Always follow recommended cleaning instructions prior to and after each experiment (see Cleaning Instructions section for more details).
- Use separate areas and dedicated equipment and supplies for:
  a. RNA sample preparation
  b. RT-LAMP reaction set-up and amplification
  c. Analysis of the RT-LAMP amplicons by end-point detection method (it is highly recommended that tubes or wells of the RT-LAMP reactions be opened in an area that is separate from the reaction set-up area to prevent environment contamination with RT-LAMP amplicons).
- Periodically clean used equipment and the working area (i.e., laboratory benchtops and workplates of the laminar flow hood) with a 10% bleach solution.
- Discard used reagents if carry-over contamination is suspected and replace them with fresh ones.
- Prevent contamination of the stock reagents by aliquoting them into the working stock volumes.
- Use aerosol-resistant filtered pipette tips only.
- Change pipette tips between components of the reaction mix, samples and technical repeats.

Cleaning instructions

- To minimize the risk of environment-borne contamination, clean the laboratory workspace and all equipment thoroughly before and after setting up each experiment. Using the following reagents in the given order:
  1. DNAZap™ PCR DNA Degradation Solution (Cat. No. AM9890)
  2. UltraPure™ DNase/RNase-Free Distilled Water (Cat. No. 10977049)
  3. 70% ethanol solution
  4. RNaseZap™ RNase Decontamination Solution (Cat. No. AM9784)
  5. 70% ethanol solution
- Wipe down instruments, pipettors, and other surfaces using DNAZap™ RNase Decontamination Wipes (Cat. No. AM9786) to avoid liquid contact with sensitive parts (e.g., electronic control modules).
- Before handling RNA samples, spray RNAZap™ RNase Decontamination Solution on gloved hands.

Prepare 50 µM SYTO™ 9 stain solution

SYTO™ 9 Green Fluorescent Nucleic Acid Stain is used for real-time detection protocols, and comes as a 5 mM stock solution.

Prepare fresh 50 µM SYTO™ 9 stain solution by making a 1:100 dilution of SYTO™ 9 Green Fluorescent Nucleic Acid Stain in nuclease-free water before every use.

Prepare RT-LAMP reaction mix

The following procedure uses volumes for a single **25-µL** reaction. **Keep the reaction mix and all reaction tubes or plates on ice to reduce non-specific amplification/background.**

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Thaw reagents</td><td>Thaw reagents on ice. Mix and briefly centrifuge all reagents to ensure homogeneity before use.
Mix target RNA by gentle tapping. Pipetting or vortexing can shear the target RNA.</td></tr>
<tr><td>2  </td><td>Prepare RT-LAMP reaction mix</td><td>a. Add the following components to each PCR tube. If preparing a master mix, multiply the volumes of components common to all reactions by the required number of reactions plus an additional 10% to account for variations in pipetting. Dispense appropriate volumes into each tube or well before adding template DNA and primers.
<table>
<tr><th>Reagent</th><th>RNA detection</th><th>No template control (NTC)</th><th>Final concentration</th></tr>
<tr><td>SuperScript™ IV RT-LAMP Master Mix (2X)</td><td>12.5 µL</td><td>12.5 µL</td><td>1 X</td></tr>
<tr><td>40 µM FIP/BIP primer</td><td>1 µL each</td><td>1 µL each</td><td>1.6 µM [2]</td></tr>
<tr><td>10 µM F3/B3 primer</td><td>0.5 µL each</td><td>0.5 µL each</td><td>0.2 µM [2]</td></tr>
<tr><td>10 µM LoopF / LoopB primer</td><td>1 µL each</td><td>1 µL each</td><td>0.4 µM [2]</td></tr>
<tr><td>fresh 50 µM SYTO™ 9 stain solution [1]</td><td>2.5 µL</td><td>2.5 µL</td><td>5 µM</td></tr>
<tr><td>Water, nuclease-free</td><td>Fill to 24 µL</td><td>Fill to 24 µL</td><td>—</td></tr>
</table>
[1] For real-time fluorescent detection only.
[2] Recommended primer concentrations, but optimization might be required to achieve the best performance. See General guidelines for primer design and Troubleshooting sections for more details.
b. Mix and then briefly centrifuge the components.</td></tr>
<tr><td>3  </td><td>Add target RNA</td><td>a. Dispense 24 µL of the prepared master mix to separate PCR tubes or individual wells of a 96-well PCR plate (e.g., MicroAmp™ Fast Optical 96-well Reaction Plate (Cat. No. 4346907) or other plate appropriate for your thermal cycler).
b. Add 1 µL of nuclease-free water to NTC reactions, then seal NTC tubes or wells to prevent cross contamination.
c. Add 1 µL of purified target RNA to RNA detection reactions.
Note: An external positive RNA control to verify RT-LAMP performance is recommended.
d. Seal the tubes or wells. For real-time fluorescent detection, use optically clear cap strips or adhesive covers only.
e. Mix reactions gently to remove any bubbles.
▪ Centrifuge briefly at1200 rpm for 2 minutes to collect material (any small bubbles at this point can be ignored since they will burst during amplification).
▪ For reactions in 96-well plates, mix on a shaker for 1 minute or by hand from side to side prior to centrifugation.</td></tr>
</table>

Automated detection options

Real-time fluorescent RT-LAMP detection

The QuantStudio™ 5 Real-Time PCR System and QuantStudio™ 6, 7, and 12K Flex Real-Time PCR Systems are compatible with the real-time RT-LAMP.

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1</td><td>Program thermal cycler</td><td>a. Program your thermal cycler with the following conditions to perform real-time RT-LAMP reactions.
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Number of cycles</th></tr>
<tr><td>Amplification</td><td>65°C</td><td>30 seconds</td><td>60</td></tr>
<tr><td>Inactivation</td><td>95°C</td><td>2 minutes</td><td>1</td></tr>
<tr><td>Melt curve</td><td>60–95°C</td><td>—</td><td>—</td></tr>
</table>
b. If using SYTO™ 9 stain, select the SYBR Green channel for signal detection.</td></tr>
<tr><td>2</td><td>Run reaction</td><td>a. Load the PCR tubes or 96-well PCR plate into the thermal cycler.
b. Start the thermal cycler program.</td></tr>
<tr><td>3</td><td>Analyze data</td><td>Analyze the data using suitable software. RT-LAMP products are long and varied in size, which tends to give a single species profile when analyzed by melting curve analysis. Specific amplification products can be separated from non-specific amplicons by differences in the respective melting curve profiles.</td></tr>
</table>

End-point detection

End-point detection can be performed with either a thermal cycler or a standard block heater.

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1</td><td>Prepare heat block</td><td>Prepare a heat block(s) for the RT-LAMP reaction.
▪ Program your thermal cycler with the following conditions.
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Number of cycles</th></tr>
<tr><td>Amplification</td><td>65°C</td><td>15–30 minutes</td><td>1</td></tr>
<tr><td>Inactivation</td><td>95°C</td><td>2 minutes</td><td>1</td></tr>
</table>
▪ Pre-heat one heat block to 65°C, and another to 95°C.</td></tr>
<tr><td>2</td><td>Run reaction</td><td>Incubate the reactions at 65°C for 15–30 minutes.</td></tr>
<tr><td>3</td><td>Inactivate reaction</td><td>Incubate the reactions at 95°C for 2 minutes to inactivate the reaction mix.</td></tr>
<tr><td>4</td><td>Analyze data</td><td>Select an appropriate read-out option for analyzing results. If RT-LAMP tubes need to be opened for analysis, it is highly recommended that they are opened in a workspace separate from the reaction set-up area. Open one tube at a time and close it quickly to minimize risks of carry-over contamination.</td></tr>
</table>

Visual detection options

Evaluation of the Color Change by Using SYBR™ Green I nucleic acid stain

After amplification, SYBR™ Green I nucleic acid stain can be used to visualize the presence of amplicons.

Step	Action	Procedure details
1	**Dilute SYBR™ Green stain**	Dilute SYBR™ Green I stain (Cat. No. S7567; 10,000X stock) 1:10 (v/v) with nuclease-free water.
2	**Stain reaction**	**a.** Add 1 µL of diluted SYBR™ Green stain to each tube. Stain negative control reactions first to prevent carry-over contamination with amplicons from positive reactions. **b.** Mix by flicking the tubes, then centrifuge the tubes and analyze the results. A bright green color indicates successful amplification, while an orange color indicates no amplification.

Evaluation by agarose gel electrophoresis

After amplification, samples can be run on agarose gels to visualize the presence of amplicons. The following procedure is for E-Gel™ Double Comb 2% Agarose Gel with SYBR™ Safe stain (Cat. No. A42348) and the E-Gel™ Power Snap Electrophoresis System (Cat. No. G8300). Other agarose gel procedures can be used according to the specific needs of the user.

Step	Action	Procedure details
1	**Prepare samples**	▪ Prepare negative control samples before test samples to minimize risk of carry-over contamination. ▪ If reactions have been previously stained using SYBR™ Green I stain, dilute 2 µL of the stained RT-LAMP reaction sample into 18 µL of nuclease-free water in order to achieve good visualization. Mix by flicking and spin down. ▪ For unstained reactions, dilute 5-fold by adding 4 µL of RT-LAMP reaction sample into 16 µL of the nuclease-free water. Mix by flicking and spin down. If a ladder-like pattern is not well visualized in positive reactions after electrophoresis, use a higher dilution ratio, e.g., a 10X dilution.
2	**Prepare DNA ladder**	Prepare the E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. 10488091) by adding 10 µL of the ladder into 10 µL of nuclease-free water for one gel lane. Gently mix and briefly spin down.
3	**Run gel**	**a.** Load 20 µL of prepared ladder and samples (starting with the negative control reactions) into the wells of the E-Gel™ agarose gel. Load negative controls and test samples in separate lanes to minimize risk of carry-over contamination. **b.** Select the appropriate program for your gel on the E-Gel™ Power Snap Electrophoresis System and run the gel. A ladder-like pattern indicates successful amplification (see **Typical results** for more details).

Typical results

Real-time fluorescent RT-LAMP detection

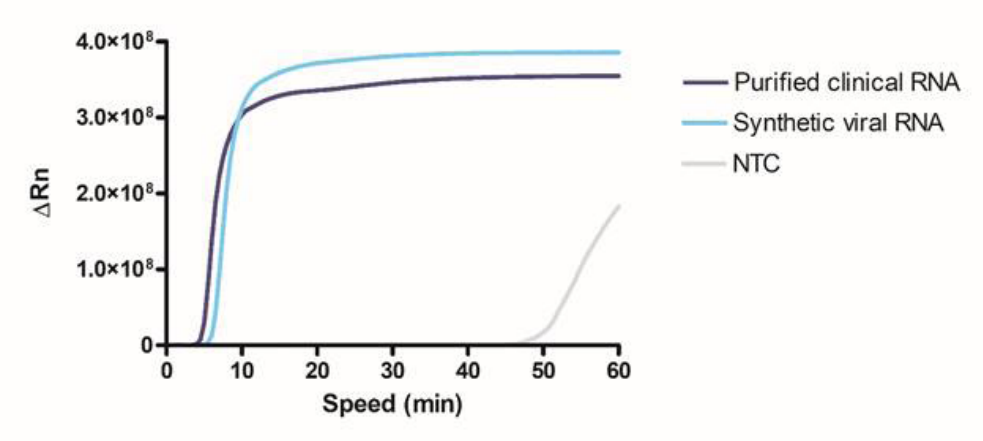

Figure 1. Detection of the viral RNA target by the real-time fluorescent RT-LAMP assay. Real-time RT-LAMP was performed using real-time PCR instrument and SYTO™ 9 stain, the reaction time was 60 minutes. Two blue curves demonstrate early amplification signal in positive reactions (< 15min). The dark blue curve represents amplification of the purified clinical RNA, and the light blue curve corresponds to the synthetic viral RNA amplification. The grey curve indicates late amplification signal in the no template control.

End-point detection

Color change by SYBR™ Green I staining and agarose gel electrophoresis

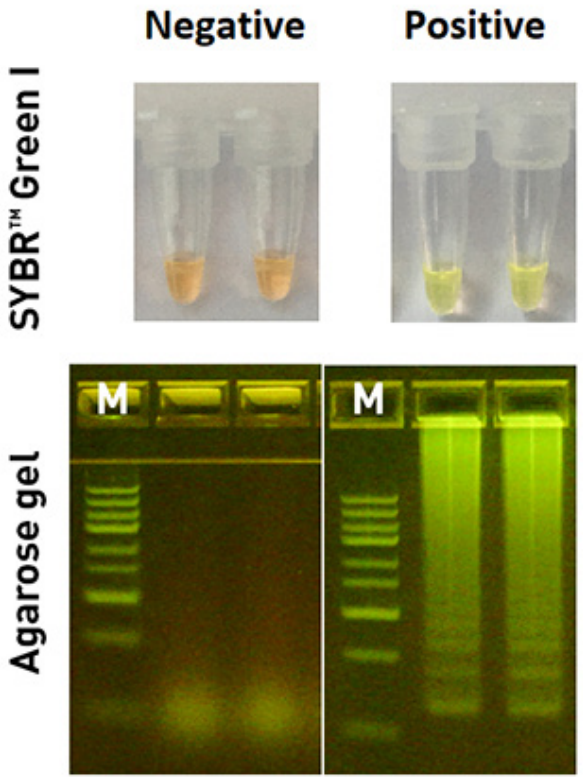

Figure 2. Visual detection of the end-point RT-LAMP reaction results.

- Upper row: Color change produced by SYBR™ Green I staining. Bright green color (right) corresponds to successful amplification of the target RNA. Orange color (left) indicates no amplification.
- Lower row: Agarose gel verification of RT-LAMP end-point detection results using a 2% E-Gel™ agarose gel with SYBR™ Safe stain. A distinct ladder-like pattern in the gel indicates a positive reaction. Absence of the ladder pattern indicates a negative result. The M lane contains the E-Gel™ 1 Kb Plus Express DNA Ladder.

Troubleshooting

Observation	Cause	Solution
False-negative	Low yield of the RT-LAMP product.	▪ Optimize primer design. ▪ Screen various concentrations of primers in order to find the most suitable ratio for specific target RNA amplification. ▪ Use HPLC-purified primers in order to improve amplification specificity. ▪ If not included, design and add Loop primers to the reaction mix. ▪ Increase the amount of target RNA. ▪ Prolong real-time reaction time from 5 minutes and end-point reaction from 15 minutes to 30 minutes. Incubation for longer than 30 minutes increases the risk of false-positive result.
	No amplification.	▪ Check primer complementarity and specificity to the target RNA. Redesign primers using appropriate software and tools. Adhere to general primer design rules. ▪ Perform RT-LAMP with validated set of primers. ▪ Mix all components of the reaction set-up prior to use. ▪ Before dispensing, gently vortex prepared reaction mix and spin it down briefly to collect the material. ▪ Use optically clear caps and adhesive covers in the real-time fluorescent detection (e.g., VersiCap Mat, 96-well, ultra clear cap strips (Cat. No. AB1820), Ultra Clear qPCR Caps (Cat. No. AB0866), MicroAmp™ Optical 8-Cap Strips (Cat. No. 4323032), and MicroAmp™ Optical Adhesive Film (Cat No. 4360954). ▪ Use freshly prepared 50 µM stock solution of SYTO™ 9 Nucleic Acid Stain. ▪ If using SYTO™ 9 Nucleic Acid Stain, check that the SYBR Green channel (reporter) is selected for the signal detection. ▪ Use external positive RNA control to check performance of the RT-LAMP reaction. ▪ Check quality of the purified target RNA. ▪ Minimize the number of freeze-thaw cycles of the target RNA in order to avoid degradation. ▪ Prevent RNase contamination by following cleaning instructions and Good Laboratory Practice (GLP) guidelines. RNaseZap™ RNase Decontamination Solution is recommended. ▪ Use nuclease-free water. ▪ Store RNA at –80°C ▪ Keep both reaction mix and the target RNA on ice during reaction set-up, ▪ Mix the target RNA gently by tapping instead of vortexing or pipetting to prevent RNA shearing.
	Non-specific amplification due to the exogenous RNA/DNA contamination.	▪ Prepare RT-LAMP reactions in the specially designated workplace with dedicated equipment and supplies. ▪ Use sterile filtered pipette tips only. ▪ Always wear gloves while performing the experiment.

Troubleshooting

Observation	Cause	Solution
False-positive result	Environment-borne and/or carry-over contamination.	▪ Follow recommended cleaning instructions before and after each experiment. ▪ Do not open tubes or wells after amplification in the RT-LAMP reaction set-up space. ▪ Analyze RT-LAMP amplicons in the workspace separate from the reaction set-up area if performing end-point detection. ▪ If contamination is observed, prepare negative controls in the area separate from adding test samples. ▪ Seal NTC tubes or wells before adding target RNA. ▪ Replace potentially contaminated reagents with new ones. ▪ Systematically clean used equipment and the working area with a 10% bleach solution. ▪ If equipment contamination is suspected, clean the heating block of the thermal cycler or block heater. ▪ Use freshly opened nuclease-free water during each experiment. ▪ If a false positive signal is observed in NTC reactions, shorten the reaction time (≤15 minutes).

Related Products and equipment

Product	Cat. No.
DNAZap™ PCR DNA Degradation Solutions	AM9890
UltraPure™ DNase/RNase-Free Distilled Water	10977049
RNaseZap™ RNase Decontamination Solution	AM9784
RNaseZap™ RNase Decontamination Wipes	AM9786
QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1 mL	A28138
QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1 mL, laptop	A28568
QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1 mL, desktop	A28573
QuantStudio™ 6 Flex Real-Time PCR System, 96-well Fast, desktop	4485697
QuantStudio™ 6 Flex Real-Time PCR System, 96-well Fast, laptop	4485699
QuantStudio™ 7 Flex Real-Time PCR System, 96-well Fast, desktop	4485693
QuantStudio™ 7 Flex Real-Time PCR System, 96-well Fast, laptop	4485698
QuantStudio™ 12K Flex Real-Time PCR System, Fast 96-well block, desktop	4471088
QuantStudio™ QuantStudio™ 12K Flex Real-Time PCR System, Fast 96-well block, laptop	4471080
Water, nuclease-free	R0581
MicroAmp™ Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL	A30588
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
VersiCap Mat, 96-well, ultra clear cap strips	AB1820
Ultra Clear qPCR Caps, strips of 8	AB0866
MicroAmp™ Optical 8-Cap Strips	4323032
MicroAmp™ Optical Adhesive Film	4360954
SYBR™ Green I Nucleic Acid Gel Stain, 10,000X concentrate in DMSO	S7567
E-Gel™ Double Comb Agarose Gels with SYBR™ Safe DNA Gel Stain, 2%	A42348
E-Gel™ 1 Kb Plus Express DNA Ladder	10488091
E-Gel™ Power Snap Electrophoresis System	G8300

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