# RT-LAMP protocol for RNA-based amplification of viral pathogens, including SARS-CoV-2

Fast and simple RNA target amplification using Bsm DNA Polymerase and SuperScript IV Reverse Transcriptase

## Materials

1. Powder-free disposable gloves
2. Sterile, nuclease-free tubes (0.2 and 1.5 mL) and pipette tips (10 or 20, 200, and 1,000 µL)
3. Pipettes (P10 or P20, P200, and P1000)
4. Ice bucket
5. Thermal cycler or heat block, vortex mixer, microcentrifuge, and electrophoresis system (optional)

## Preparation

- Before you begin, thaw all reaction components on ice.
- If analyzing with an Invitrogen™ E-Gel™ Power Snap Electrophoresis System (Cat. No. G8300) or any other system that requires opening of reverse-transcription loop-mediated isothermal amplification (RT-LAMP) reaction tubes, set up a secondary laboratory workspace to avoid equipment contamination with RT-LAMP amplicons.
- To minimize the risk of environmental contamination, thoroughly clean the laboratory workspace and all equipment using the following reagents in the provided order. The cleaning protocol needs to be applied prior to and after each experiment:
  1. Invitrogen™ DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890)
  2. Distilled, DNase/RNase-free water
  3. 70% ethanol solution
  4. Invitrogen™ RNase*Zap*™ RNase Decontamination Solution (Cat. No. AM9784)
  5. 70% ethanol solution
- Prior to handling RNA samples, spray RNase*Zap* RNase Decontamination Solution on gloved hands, and wipe down instruments, pipettors, and other surfaces using Invitrogen™ RNase*Zap*™ RNase Decontamination Wipes (Cat. No. AM9786) to avoid liquid contact with sensitive parts (e.g., electronic control modules).
- Additionally, use nuclease-free tubes and tips to minimize RNA degradation.

  **Note**: Find out more about preventing RNA degradation at **thermofisher.com/rnase**.

## Purification of total RNA

- For sample preparation, use the Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat. No. A42352) and follow the recommended protocol.

## Protocol

The following protocol was tested with total RNA from clinical research samples.

1. Assemble reaction tubes on ice, in the order listed below.

   **Note**: To ensure specificity, run several negative control reactions (e.g., no-template control, nontarget template control).

| Component | Volume | Final concentration | Cat. No. |
|---|---|---|---|
| **Invitrogen™ RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 U/µL stock)** | 1 µL | 1.6 U/µL | 10777019 |
| **Thermo Scientific™ 10X Bsm Buffer** | 2.5 µL | 1X | EP0691 |
| **Thermo Scientific™ $MgCl_2$ (25 mM stock)** | 4 µL | 4 mM* | R0971 |
| **Thermo Scientific™ dNTP mix (10 mM stock)** | 3.5 µL | 1.4 mM each | R0191 |
| **FIP/BIP primers (40 µM stock)** | 1 µL each | 1.6 µM | From publication [1] |
| **F3/B3 primers (10 µM stock)** | 0.5 µL each | 0.2 µM | From publication [1] |
| **LoopF/B primers (10 µM stock)** | 1 µL each | 0.4 µM | From publication [1] |
| **Thermo Scientific™ Water, nuclease-free** | 6 µL | – | R0581 |
| **Thermo Scientific™ Bsm DNA Polymerase, large fragment (8 U/µL)** | 1 µL | 0.32 U/µL | EP0691 |
| **Invitrogen™ SuperScript™ IV Reverse Transcriptase (200 U/µL)** | 1 µL | 8 U/µL | 18090050 |
| **RNA template** | 1 µL | Variable | – |
| **Total reaction volume** | **25 µL** | **–** | **–** |

* An additional 2 mM of $Mg^{2+}$ comes from the 10X Bsm Buffer. The final concentration of $Mg^{2+}$ is 6 mM.

2. Mix the tubes gently by flicking and briefly spinning down.
3. Incubate the tubes at 60°C for 50 min.
4. To inactivate the reactions, incubate at 95°C for 2 min.
5. To visualize results, either:
   a. Add 1 µL of Invitrogen™ SYBR™ Green I stain (Cat. No. S7567; 10,000X stock) diluted with nuclease-free water 1:10 (v/v) to each tube.

      **Important**: Open one tube at a time and add stain quickly. Mix by flicking the tubes. Spin down and analyze results. Green color indicates successful amplification, and orange color indicates no amplification.

   b. Run the samples on an Invitrogen™ E-Gel™ agarose gel:
      i. Start by cleaning the E-Gel Power Snap Electrophoresis system with DNA*Zap* PCR DNA Degradation Solutions, distilled, DNase/RNase-free water, and 70% ethanol as described above.

         **Recommendation**: Use an Invitrogen™ E-Gel™ Double Comb 2% Agarose Gel with SYBR™ Safe stain (Cat. No. A42348).

      ii. Prepare the Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. 10488091) by adding 10 µL of ladder into 10 µL of nuclease-free water. Gently mix and briefly spin down.

      iii. Prepare negative control samples. Then, prepare test samples by adding 5 µL of the RT-LAMP reaction sample into 15 µL of nuclease-free water. Mix by flicking and spinning down.

      iv. Transfer 20 µL of prepared ladder and samples (starting with negative controls) into the E-Gel agarose gel wells. Load negative control and test samples into separate lanes to minimize the risk of carryover contamination.

      v. Run electrophoresis for 15 min and analyze results. A ladder-like band pattern indicates successful amplification.

## Ordering information

| Product | Cat. No. |
|---|---|
| Bsm DNA Polymerase, large fragment (8 U/µL) | EP0691 |
| SuperScript IV Reverse Transcriptase | 18090050 |
| $MgCl_2$ (magnesium chloride) (25 mM) | R0971 |
| dNTP Mix (10 mM each) | R0191 |
| Water, nuclease-free | R0581 |
| DNA*Zap* PCR DNA Degradation Solutions | AM9890 |
| RNase*Zap* RNase Decontamination Solution | AM9784 |
| RNase*Zap* RNase Decontamination Wipes | AM9786 |
| RNaseOUT Recombinant Ribonuclease Inhibitor | 10777019 |
| SYBR Green I Nucleic Acid Gel Stain, 10,000X concentrate in DMSO | S7567 |
| E-Gel Agarose Gels with SYBR Safe DNA Gel Stain, 2% | A42135 |
| E-Gel 1 Kb Plus Express DNA Ladder | 10488091 |
| MagMAX Viral/Pathogen Nucleic Acid Isolation Kit | A42352 |

Custom DNA primers are available in several formats and are synthesized using our state-of-the-art platforms with rigorous quality control. For more information on designing and ordering primers, go to **thermofisher.com/primers**.

### Reference

1. Park GS, Ku K, Baek SH et al. (2020) Development of reverse transcription loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *J Mol Diagn* 22(6):729-735. **doi:10.1016/j.jmoldx.2020.03.006**.

Find out more at **thermofisher.com/lamp**

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