SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

NEB #E2019S

96 reactions

Version 2.0_11/21

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Kit Components:

Reagents are shipped on ice packs and should be placed at -20°C upon receipt. All components will freeze at -20°C and must be thawed before use. Thaw components at room temperature and then place on ice or at 4°C during use. Store materials at -20°C after use. LAMP primers and Positive Control should be gently vortexed before reaction setup and WarmStart Colorimetric LAMP 2X Master Mix with UDG (NEB #M1804) should be mixed well by vortexing to ensure any precipitation formed during freeze/thaw is resuspended.

WarmStart® Colorimetric LAMP 2X Master Mix with UDG (2X)

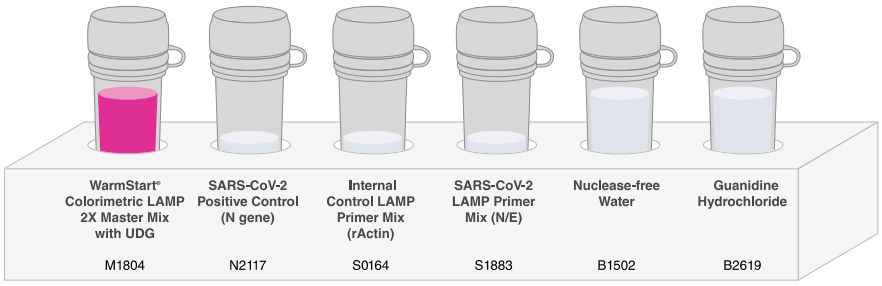
SARS-CoV-2 Positive Control (N gene) (12.5X)

Internal Control LAMP Primer Mix (rActin) (10X)

SARS-CoV-2 LAMP Primer Mix (N/E) (10X)

Nuclease-free Water

Guanidine Hydrochloride (0.4 M) (10X)



Required Equipment/Materials Not Included

Disposable powder-free gloves and any additional PPE required

P2/P10, P200, and P1000 aerosol barrier tips

Sterile, nuclease-free 1.5 ml microcentrifuge tubes

Sterile, nuclease-free 2.0 ml, 5.0 ml, or 15 ml tubes

0.2 ml PCR reaction tube strips with separated tubes and lids (e.g., VWR 20170-004) or attached caps (e.g., VWR 20170-010) or 96-well PCR reaction plates with 8-cap strips

Racks for 1.5 ml microcentrifuge tubes and 96-well 0.2 ml PCR reaction tubes

Cooler rack for 1.5 microcentrifuge tubes and 96-well 0.2 ml PCR reaction tubes

Acceptable surface decontaminants, for example: 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)

Laboratory marking pen

Appropriate disposal containers

White paper or light background for optimal visualization of colorimetric reaction (e.g., typical printer paper)

Micropipettes (2 or 10 µl, 200 µl and 1,000 µl), Multichannel Micropipettes (5-50 µl)

-20°C Freezer (frost-free or nonfrost), 4°C Refrigerator

Thermocycler, heat block or device that can be set to 65°C

PCR Work Station [UV lamp; Laminar flow (Class 100 HEPA filtered)], Vortex Mixer, Tabletop Microcentrifuge

Warnings and Precautions

- Visualization of color analysis should be performed in a separate location from assay setup
- **DO NOT** open assay tubes following incubation at 65°C. Visualize assay results, record results and dispose of assays immediately upon completion.
- Waste should be disposed of in compliance with local, state and federal regulations
- Always use pipette tips containing aerosol barriers that are sterile and free of DNases and RNases
- Appropriate safety procedures should be followed at all times
- Reagents must be stored at -20°C when not in use

Introduction

The SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit is a rapid colorimetric assay for *in vitro* detection of SARS-CoV-2 RNA that relies on loop-mediated isothermal amplification (LAMP). The WarmStart Colorimetric LAMP Master Mix with UDG combines WarmStart *Bst* 2.0 DNA Polymerase and WarmStart RTx Reverse Transcriptase to enable nucleic acid amplification at a single reaction temperature. The 2X colorimetric master mix also contains a weakly buffered solution and a pH-sensitive dye that changes color upon acidification. Amplification in the presence of target nucleic acids results in the production of protons that cause a decrease in pH, resulting in a clear visual color change from pink to yellow that is easily detectable by eye.

The WarmStart activated enzymes allow dual control of enzyme activity by reversible, aptamer-based inhibition (SOMAmers®). This temperature-dependent activation helps prevent undesired non-specific priming and extension prior to isothermal incubation at 65°C, providing added security for setting up reactions at room temperature.

The colorimetric mix is enabled with carryover prevention (dUTP/UDG). It is formulated with a mixture of dTTP and dUTP. This ensures both efficient isothermal amplification as well as the incorporation of dU into the reaction products. LAMP products containing dU serve as a substrate for Antarctic Thermolabile Uracil DNA Glycosylase (UDG) present in the master mix, allowing carryover contamination prevention. Antarctic Thermolabile UDG will be completely inactivated upon isothermal incubation at 65°C. Because LAMP can generate large quantities of DNA in very short periods of time, best practices to reduce contamination involve not opening LAMP reactions post amplification.

The SARS-CoV-2 LAMP Primer Mix provided within the kit contains a mixture of primers that target both the nucleocapsid (N) gene and envelope (E) gene of SARS-CoV-2. The primer sets perform well individually but mixing them improves detection (https://pubmed.ncbi.nlm.nih.gov/32635743/). Upon successful amplification, the reaction mix will change color from pink to yellow, or yellow/orange color. An internal control (IC) primer set that amplifies rActin is included to ensure the absence of inhibition from human nucleic acid templates. A positive control (PC) template containing the N-gene is also included. Guanidine hydrochloride has been shown to improve colorimetric LAMP at a concentration of 40 mM and is provided to supplement in the reaction for any samples that do not

already contain guanidine. Guanidine concentrations up to 60 mM (final concentration at 1X) are tolerated. A sample can only be judged for the presence or absence of SAR-CoV-2 RNA if all reactions are pink prior to incubation and post incubation, the NTC reaction is pink, the PC reaction is yellow and the IC reaction is yellow.

Sample Compatibility

- Sample input should be purified total nucleic acid eluted in nuclease-free water for best results
- Material stored in TE or similar elution buffer should be kept to less than 5 µl (20% v/v) of the final reaction volume as excess buffer may inhibit the color change
- Up to 1 µl (4% v/v) of transport media (UTM or VTM) may be used without impacting the colorimetric reaction
- Acidic samples may immediately turn the colorimetric LAMP reaction orange or yellow upon addition. Try adjusting the sample pH to ~8.0 prior to addition to the reaction.
- Samples should not contain SDS as it is a strong inhibitor of amplification
- Small amounts of Tween® 20 or Triton™ X-100 (≤ 0.2%) are tolerated
- If samples contain a source of guanidine, ensure that the final concentration of guanidine remains less than 60 mM to avoid inhibiting the LAMP reaction

SARS-CoV-2 Rapid Colorimetric LAMP Assay Protocol

1. Thaw WarmStart Colorimetric LAMP 2X Master Mix with UDG, LAMP Primer Mixes, Positive Control, and Guanidine Hydrochloride at room temperature. Once thawed, place on cold rack at 4°C or on ice.

2. Mix each component thoroughly by gently vortexing. Ensure that any precipitation in the WarmStart Colorimetric LAMP 2X Master Mix with UDG (NEB #M1804) is completely resuspended. Briefly centrifuge all components to collect liquid at the bottom of the tubes before opening.

3. A total of four reactions are required for each nucleic acid sample being evaluated:
    #1. No Template Control (NTC)
    #2. Positive Control
    #3. Internal Control (rActin)
    #4. SARS-CoV-2 Test Sample

    The SARS-CoV-2 LAMP Primers will be used for the NTC, Positive Control, and SARS-CoV-2 sample reactions. The Internal Control confirms the activity of provided reagents, proper sample handling and the presence of human nucleic acid template using the Internal Control LAMP Primer Mix (rActin).

    An overview of the reaction setup for one sample is described in the table below. Do not assemble the reactions until Step 4.

COMPONENT	#1. NO TEMPLATE CONTROL	#2. POSITIVE CONTROL	#3. INTERNAL CONTROL	#4. SARS-CoV-2 TEST SAMPLE
WarmStart Colorimetric LAMP 2X Master Mix with UDG	12.5 µl	12.5 µl	12.5 µl	12.5 µl
SARS-CoV-2 Positive Control (N gene)	–	2.0 µl	–	–
Internal Control LAMP Primer Mix (rActin)	–	–	2.5 µl	–
SARS-CoV-2 LAMP Primer Mix (N/E)	2.5 µl	2.5 µl	–	2.5 µl
Nuclease-free Water	7.5 µl	5.5 µl	5.5 µl	5.5 µl
Guanidine Hydrochloride*	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Sample Nucleic Acid**	–	–	2.0 µl	2.0 µl

* Guanidine hydrochloride should be added to a final concentration of 40 mM only when samples do not already contain guanidine. If guanidine will be carried into the reaction with the input material, this volume should be replaced with nuclease-free water.

** For information regarding sample compatibility with colorimetric LAMP, please see Sample Compatibility section on previous page.

4. Prepare the components indicated below at room temperature for the number of samples (*n*) being evaluated. Volumes include 10% overage to accommodate transfer loss from pipetting.

   SARS-CoV-2 LAMP Reaction Mix (for 3 of 4 reactions per sample):

COMPONENT	VOLUME FOR ONE SAMPLE	VOLUME FOR *n* SAMPLES	VOLUME FOR 24 SAMPLES
WarmStart Colorimetric LAMP 2X Master Mix with UDG	41.25 µl	41.25 µl x *n*	990.0 µl
Nuclease-free Water	18.15 µl	18.15 µl x *n*	435.6 µl
SARS-CoV-2 LAMP Primer Mix (N/E)	8.25 µl	8.25 µl x *n*	198.0 µl
Guanidine Hydrochloride*	8.25 µl	8.25 µl x *n*	198.0 µl

* Guanidine hydrochloride should be added to a final concentration of 40 mM only when samples do not already contain guanidine. If guanidine will be carried into the reaction with the input material, this volume should be replaced with nuclease-free water.

   Internal Control LAMP Reaction Mix (for 1 of 4 reactions per sample):

COMPONENT	VOLUME FOR ONE SAMPLE	VOLUME FOR *n* SAMPLES	VOLUME FOR 24 SAMPLES
WarmStart Colorimetric LAMP 2X Master Mix with UDG	13.75 µl	13.75 µl x *n*	330.0 µl
Nuclease-free Water	6.05 µl	6.05 µl x *n*	145.2 µl
Internal Control LAMP Primer Mix (rActin)	2.75 µl	2.75 µl x *n*	66.0 µl
Guanidine Hydrochloride*	2.75 µl	2.75 µl x *n*	66.0 µl

* Guanidine hydrochloride should be added to a final concentration of 40 mM only when samples do not already contain guanidine. If guanidine will be carried into the reaction with the input material, this volume should be replaced with nuclease-free water.

   Aliquot 23 µl of the LAMP reaction mixes in a PCR strip tube or 96-well PCR plate at room temperature. Pipette 2.0 µl of Nuclease-free Water, SARS-CoV-2 Positive Control, or Sample Nucleic Acid into the reaction according to the table below.

COMPONENT	#1. NO TEMPLATE CONTROL	#2. POSITIVE CONTROL	#3. INTERNAL CONTROL	#4. SARS-CoV-2 TEST SAMPLE
SARS-CoV-2 LAMP Reaction Mix (prepared above)	23 µl	23 µl	–	23 µl
Internal Control LAMP Reaction Mix (prepared above)	–	–	23 µl	–
Nuclease-free Water	2.0 µl	–	–	–
SARS-CoV-2 Positive Control (N gene)	–	2.0 µl	–	–
Sample Nucleic Acid	–	–	2.0 µl	2.0 µl

5. Gently vortex reactions to mix well. Verify that all starting reactions are pink. A color change to yellow or orange upon the addition of sample nucleic acid for the internal control or SARS-CoV-2 test sample reaction indicates the input material is incompatible with the assay. It should not be interpreted to signify the presence of target nucleic acid. See sample combability section above for additional information. If the NTC or PC reactions turn yellow or orange, repeat assay set up from Step 4.

6. Place reactions directly into a pre-heated thermocycler or heat block set to 65°C. Incubate at 65°C for 30 minutes. For instruments with a heated lid, we recommend its use at 105°C. NOTE: For very low copy samples or maximum sensitivity, time may need to be extended to 40 minutes.

7. Allow reactions to cool from 65°C by placing at room temperature for 5 min for improved color contrast, or place tubes on ice for 5 seconds. **DO NOT** open the reaction vessels. Inspect reaction tubes for color and record results. NOTE: results can be read for up to 6 hours after reactions have been run.

8. A sample can only be judged for the presence or absence of SAR-CoV-2 RNA if the NTC reaction is pink, the PC reaction is yellow and the IC reaction is yellow. (See diagram below for interpretation of results).

9. Discard all completed reactions as medical waste.

Usage Notes:

Carryover Contamination Prevention

LAMP is a sensitive method that generates large quantities of DNA, and contamination in new LAMP assays with products from previous amplification reactions can cause a variety of issues, such as false positive results and a decrease in sensitivity. The best way to prevent this "carryover" contamination is to practice good laboratory procedures and avoid opening the reaction vessel post amplification. However, to accommodate situations where additional anti-contamination measures are desired, WarmStart Colorimetric LAMP Master Mix with UDG contains a mixture of dUTP/dTTP that results in the incorporation of dU into the DNA product during amplification. Antarctic Thermolabile Uracil DNA Glycosylase (UDG) present in the colorimetric mix will eliminate previously-amplified uracil-containing products by excising the uracil base to produce a non-amplifiable DNA product. The use of a thermolabile UDG is important, as quick and complete inactivation of the UDG is required to prevent destruction of newly synthesized LAMP products. To maximize elimination of contaminating products, set up colorimetric LAMP experiments at room temperature or include a 10 minute incubation step at 25°C before isothermal incubation.

Reaction Setup and Isothermal Incubation

Due to the dual WarmStart feature of the Colorimetric LAMP Master Mix with UDG, reactions may be set up at room temperature. Room temperature set up will also allow UDG to destroy previously generated dU-containing LAMP products.

We recommend a final reaction volume of 25 µl.

For maximum sensitivity, go directly from room temperature to a preheated block set at 65°C. Reactions should not be allowed to sit on a block as it warms to 65°C.

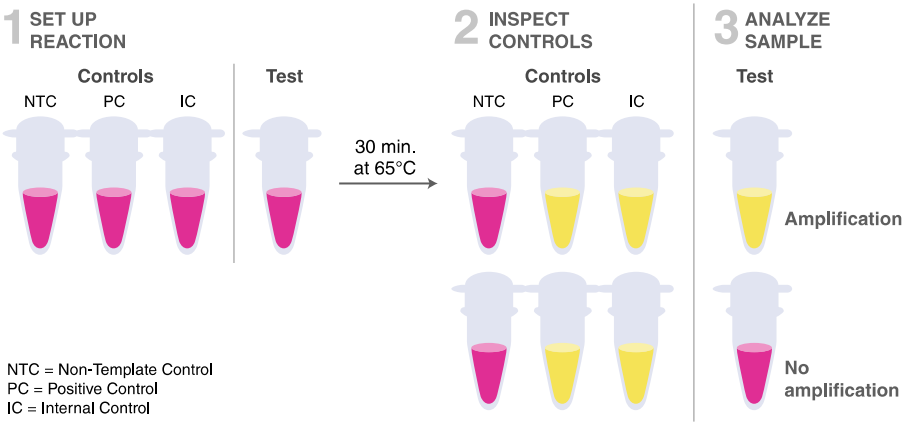
Troubleshooting Guide

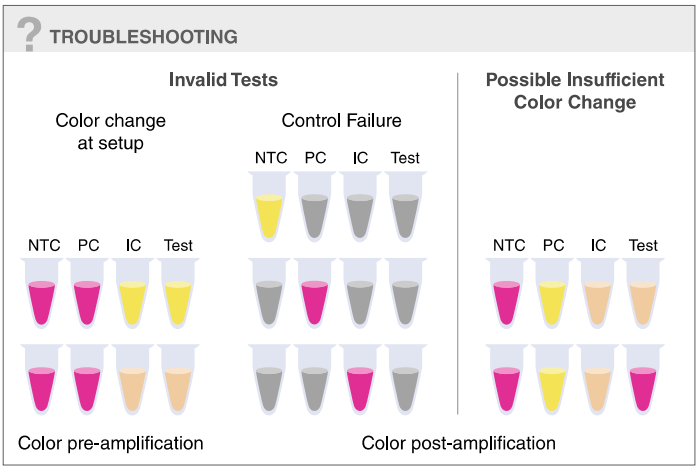
Note: For additional assistance please refer to the product FAQ's at www.neb.com/E2019

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Colorimetric LAMP reaction is orange prior to amplification	Nucleic acid sample input is incompatible	• Dilute sample in nuclease-free water or adjust the pH to ~8.0 prior to addition to the reaction
	Repeated exposure of the colorimetric LAMP master mix to atmosphere causes a slight pH drop	• Avoid extended air exposure by closing the cap and storing the master mix in the vial provided at -20°C
Positive Control reaction fails to turn yellow following incubation at 65°C	Improper pipetting during colorimetric LAMP set-up	• Ensure proper pipetting techniques are used.
	Poor mixing of reagents during set-up	• Make sure all reagents are properly mixed after thawing • Ensure reactions are properly mixed prior to incubation
NTC reaction turns yellow following incubation at 65°C	Reagents are contaminated with the positive control	• Replace all stocks and reagents • Clean equipment and setup area with 10% chlorine bleach
	Improper pipetting during colorimetric LAMP set-up	• Ensure proper pipetting techniques are used
Internal Control reaction fails to turn yellow following incubation at 65°C	Improper pipetting during colorimetric LAMP set-up	• Ensure proper pipetting techniques are used
	Sample nucleic acid is incompatible with colorimetric LAMP	• Dilute sample in nuclease-free water or adjust the pH to ~8.0 prior to addition to the reaction
	Sample nucleic acid is isolated from a non-human source	• Use human nucleic acid input • Design LAMP primers for your specific sample source
	Poor mixing of reagents during set-up	• Make sure all reagents are properly mixed after thawing • Ensure reactions are properly mixed prior to incubation
	RNA template is contaminated or degraded	• Prepare high quality RNA

Interpretation of Results

Colorimetric LAMP Reaction Color Guide





SARS-CoV-2 LAMP Primer Sequences (5´→ 3´)

Gene E Primer Set	Sequence
E1-F3	TGAGTACGAACTTATGTACTCAT
E1-B3	TTCAGATTTTTAACACGAGAGT
E1-FIP	ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG
E1-BIP	TTGCTAGTTACACTAGCCATCCTTAGGTTTTACAAGACTCACGT
E1-LF	CGCTATTAACTATTAACG
E1-LB	GCGCTTCGATTGTGTGCGT

Gene N2 Primer Set	Sequence
N2-F3	ACCAGGAACTAATCAGACAAG
N2-B3	GACTTGATCTTTGAAATTTGGATCT
N2-FIP	TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC
N2-BIP	CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA
N2-LF	GGGGGCAAATTGTGCAATTTG
N2-LB	CTTCGGGAACGTGGTTGACC

Internal Control LAMP Primer Sequences: rActin (5´→ 3´)

rActin Primer Set	Sequence
ACTB-F3	AGTACCCCATCGAGCACG
ACTB-B3	AGCCTGGATAGCAACGTACA
ACTB-FIP	GAGCCACACGCAGCTCATTGTATCACCAACTGGGACGACA
ACTB-BIP	CTGAACCCCAAGGCCAACCGGCTGGGGTGTTGAAGGTC
ACTB-LF	TGTGGTGCCAGATTTTCTCCA
ACTB-LB	CGAGAAGATGACCCAGATCATGT

Ordering Information

NEB #	PRODUCT	SIZE
E2019S	SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit	96 reactions

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
M1804S/L	WarmStart Colorimetric LAMP Master Mix with UDG	100/500 reactions
T2010S	Monarch® Total RNA Miniprep Kit	50 preps

Revision History

REVISION #	DESCRIPTION	DATE
1.0		7/20
2.0	Update legal text	11/21

This product is not available for sale in China (including Hong Kong and Macau), Japan and Taiwan. For questions, please contact busdev@neb.com.

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