## Summary

- Accurate—able to detect RNA of emerging SARS-CoV-2 variants
- Fast time-to-results—LAMP workflow can be completed in approximately 30 min without specialized equipment or advanced training

### Introduction

SARS-CoV-2 surveillance testing continues to be paramount, especially given the emergence of viral variants and the proportion of asymptomatic infections [1,2]. To address the scale and urgency of this required surveillance, the Invitrogen™ Colorimetric ReadiLAMP™ Kit can be employed for rapid, robust, and specific detection of SARS-CoV-2 RNA in saliva, nasal swab, and nasopharyngeal swab sample types. The Colorimetric ReadiLAMP Kit offers a simple visual readout—if a target is not present, the reaction mixture stays purple (negative), and if a target is present, the reaction mixture turns blue (positive). No special equipment is needed to perform or analyze the results—only a 65°C heat block or a thermal cycler, such as the Applied Biosystems<sup>™</sup> VeritiPro<sup>™</sup> Thermal Cycler, is required. The Colorimetric ReadiLAMP Kit is based on loop-mediated isothermal amplification (LAMP) primers that are complementary to more than 98% of deposited SARS-CoV-2 genomes from the Global Initiative on Sharing Avian Influenza Data (GISAID) [3], including those annotated as Omicron variant genomes. Herein, the detection of RNA from the Delta and Omicron variants of SARS-CoV-2 is validated to ensure continued surveillance confidence and detection accuracy.

### Materials and methods

## SARS-CoV-2 RNA controls

Thermo Scientific™ AcroMetrix™ Coronavirus 2019 (COVID-19) RNA Control (Cat. No. 954519) is a complete SARS-CoV-2 genomic RNA template and served as the positive control for the LAMP reactions. This control was quantified by the manufacturer using droplet-based digital PCR (ddPCR) and used directly without manipulation. SARS-CoV-2 Delta and Omicron variant RNA controls were purchased as pooled *in vitro*—transcribed fragments that cover >99.9% of their respective viral variant genomes (Twist Bioscience; Table 1). These controls were provided at a concentration of approximately 1 x 10<sup>6</sup> copies/µL and were diluted 1:20,000 in nuclease-free water. All controls and dilutions were stored as recommended by the manufacturer and were discarded after two freeze—thaw cycles.

Table 1. Synthetic SARS-CoV-2 RNA controls from Twist Bioscience.

GISAID ID	GISAID name
EPI_ISL_1544014	India/MH-NCCS- P1162000182735/2021
EPI_ISL_2695467	Portugal/PT9543/2021
EPI_ISL_2693246	USA/WA-CDC- UW21061750277/2021
EPI_ISL_6841980	Hong Kong/HKU-211129- 001/2021
EPI_ISL_7190366	Australia/QLD2568/2021
EPI_ISL_7718520	England/MILK-2DF642C/2021
	EPI_ISL_1544014 EPI_ISL_2695467 EPI_ISL_2693246 EPI_ISL_6841980 EPI_ISL_7190366





# SARS-CoV-2 detection using the Colorimetric ReadiLAMP Kit

The colorimetric assay was performed as recommended using the purified RNA sample workflow, where each control was evaluated in triplicate LAMP reactions. Nuclease-free water served as the no-template control (NTC). Positive controls included 200 copies of AcroMetrix COVID-19 RNA Control per 25  $\mu$ L reaction. For all assays of the viral variants, approximately 250 copies of synthetic RNA were included per 25  $\mu$ L reaction.

#### Results and conclusions

The Colorimetric ReadiLAMP Kit allows users to visually interpret LAMP reactions as positive (blue) or negative (purple) for the presence of SARS-CoV-2. In this study, we demonstrated that the Colorimetric ReadiLAMP Kit can successfully detect SARS-CoV-2; all reactions with SARS-CoV-2 RNA resulted in a positive blue color, while NTC reactions remained the negative purple color (Figure 1). Based on these results and the *in silico* analysis of deposited SARS genomic sequences, the Colorimetric ReadiLAMP Kit can successfully detect SARS-CoV-2, including the Delta and Omicron variants. Successful detection of the Omicron variant includes both sublineages BA.1 and BA.2 (often referred to as the "stealth Omicron variant"). Surveillance testing using the Colorimetric ReadiLAMP Kit provides accurate and specific SARS-CoV-2 detection, even as this virus continues to evolve.

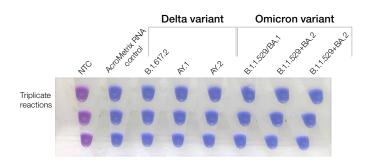


Figure 1. The Colorimetric ReadiLAMP Kit successfully detects RNA from the Delta and Omicron variants of SARS-CoV-2.

## Ordering information

Product	Quantity	Cat. No.
Colorimetric ReadiLAMP Kit, SARS-CoV-2	100 reactions	A52539
	1,000 reactions	A52544
VeritiPro Thermal Cycler, 96 well	1 ea	A48141

## References

- 1. Fontanet A, Autran B, Lina B et al. (2021) SARS-CoV-2 variants and ending the COVID-19 pandemic. Lancet 397(10278):952-954. doi:10.1016/S0140-6736(21)00370-6
- 2. Viana R, Moyo S, Amoako D et al. (2022) Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature 603(7902):679-686. doi:10.1038/s41586-022-04411-y
- 3. Khare S, Gurry C, Freitas L et al. (2021) GISAID's role in pandemic response. China CDC Weekly 3(49):1049-1051. doi:10.46234/ccdcw2021.255



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