SARS-CoV-2
Mutation Detection
N501Y

SARS-CoV-2 Mutation Detection-N501Y Assay is an isothermal RT-LAMP laboratory research use assays for detection of spike gene mutation N501Y found in the
SARS-CoV-2 lineages B.1.1.7, B.1.351, P1

Cat. no.: X-KIT-102

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LABORATORY RESEARCH USE ONLY

CONTENTS

1 INTENDED USE

SARS-CoV-2 Mutation Detection – N501Y assay is an isothermal RT-LAMP laboratory research use assay for detection of spike gene mutation N501Y found in the SARS-CoV-2 lineages of concern B.1.1.7, B.1.351 and P.1.

Genetic variants of SARS-CoV-2 RNA can be found in the liquid from upper or lower respiratory tracts of infected individuals. Samples can be obtained from isolated viral RNA from naso- or oropharyngeal, swabs. Infection with any variant of SARS-CoV-2 can occur without showing any symptoms.

Negative RT-LAMP results do not exclude present or hinder future infection with SARS-CoV-2 virus or any genetic variations and the result should always be combined with clinical observations, patient history, and epidemiological information.

SARS-CoV-2 Variant Detection Assays are intended for use by science and health professionals or qualified laboratory personnel specifically instructed and trained in molecular testing techniques as well as proficient in handling biological samples.

2 SUMMARY AND TEST PRINCIPLE

There is an urgent need for high throughput and rapid detection of SARS-CoV-2 infections and identification of variants of concern. To address this challenge, BioThinx has developed a new line of research use RT-LAMP assay systems which can detect marker mutations found in SARS-CoV-2 variants of concern: cluster B.1.1.7, cluster B.1.351, cluster P.1. An additional assay is intended for SARS-CoV-2 general detection without mutation differentiation. All assays come in an easy to handle high-throughput format.

Molecular testing uses one-step reverse transcription and loop-mediated isothermal amplification (RT-LAMP) method.

The primers of this LAMP assay have been designed to target marker mutation **N501Y** within the spike protein gene found in SARS-CoV-2 lineages **B.1.1.7** (British Variant), **B.1.351** (South African Variant) and **P.1** (Brazilian Variant).

Initially in the United Kingdom (UK), a new variant of SARS-CoV-2 emerged with a large number of mutations. This variant is associated with increased transmissibility. Beside other mutations, the UK B.1.1.7 lineage has multiple mutations in the spike glycoprotein's gene S including HV 69-70 deletion and **N501Y**.

Initially in South Africa, a variant of SARS-CoV-2 (B.1.351) emerged. This variant shares some mutations with B.1.1.7. and has multiple mutations in the spike protein, including K417N, E484K, **and N501Y**. There is some evidence to indicate that one of the spike protein mutations, E484K, may affect neutralization by some polyclonal and monoclonal antibodies.

Initially in Brazil, a variant of SARS-CoV-2 (P.1) emerged. This variant has 17 unique mutations, including three in the receptor binding domain of the spike protein (K417T, E484K, and **N501Y)**. There is evidence that some of the mutations in the P.1 variant may affect its transmissibility and antigenic profile.

3 SAFETY NOTES

This product is for LABORATORY RESEARCH USE ONLY, not for diagnostic, therapeutic, drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.

Treat all biological specimens, including used assay tubes and transfer pipettes, as if capable of transmitting infections agents

Follow your institution's safety procedures for working with chemicals and handling biological samples.

Wear laboratory coats, laboratory gloves and eye protection when handling biological samples and reagents.

Remove gloves and wash hands thoroughly after handling samples and reagents.

Do not open the tubes or unseal wells during or after amplification.

Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with all local, state and federal regulations for hazardous material.

4 MATERIALS

Loop amplification reaction tubes. Clear PCR single Cap 8-tube strips 200 µl, optical clear flat cap, high profile, cut able, filled with dried, stabilized loop amplification and detection mix.	5 strips
LAMP reaction buffer	1 vial, 1000 µl
Negative Control	1 vial, 50 µl
Positive Control	1 vial, 50 µl

5 TECHNICAL DATA

Sample:	purified RNA
Sample volume:	2 µl
Total incubation:	45 min at 65 °C
Sensitivity:	< 500 targets/µl
Shipment	ambient temperature
Storage:	-20 °C
Shelf life:	12 months after manufacturing or until the expiration date
Package size:	40 tests

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6 LOOP AMPLIFICATION PROCEDURE

6.1 INSTRUMEMTATION AND MATERIALS REQUIRED

- PCR instrument or incubator programmable at 65 °C constant temperature
- Pipettes for 1-20 µl
- Pipette tips with aerosol filters

- Collection Kits:
  Nasopharyngeal Swab
  Nasal Swab

- Extraction Kit:
  Viral DNA/RNA Extraction Kit

6.2 SAMPLE COLLECTION / PREPARATION

Preferentially, use the same RNA sample from which SARS-CoV-2 was detected, to genotype it for mutations. Ineffective or inappropriate sample collection can result in false test results. Extracted RNA should always be stored at -70°C or lower in an RNase free environment.

6.3 REAGENT PREPARATION / STORAGE

The SARS-COV-2 Mutation Detection Assay is stabilized and shipped at ambient temperature. Reagents must be stored at -20 °C upon arrival.

The reaction tubes are prefilled with LAMP reaction mixture and are supplied in a convenient dry stabilized format, containing all buffer reagents, primers, dNTPs and enzymes to perform the amplification and detection.

LAMP reaction buffer, positive and negative control are liquid and ready to use.

When stored refrigerated at -20 °C the components are stable for at least 30 days after opening or until the expiration date printed on the labels.
Remaining reaction tubes should be stored refrigerated at -20 °C protected from moisture; store together with desiccant in the resealable bag (ZipLoc).

6.4 TECHNICAL NOTES

External control RNA should routinely be assayed as unknowns to check performance of the reagents and the assay.

Use disposable filter tips to dispense reaction buffer and samples. To avoid carryover contamination, change the tip between each sample.

6.5 ASSAY PROCEDURE

Strictly follow the procedure and Good Laboratory Practice.

Please adhere strictly to the sequence of pipetting steps provided in this protocol. Observe the guidelines for performing quality control in medical laboratories by assaying external controls.

All reagents should be stored refrigerated at -20 °C in their original container.

Do not exchange kit components from different lots. The expiration dates stated on the labels of the shipping container and all vials have to be observed. Do not use kit components after their expiration dates.

Reagents removed from refrigerator should be brought to room temperature
Prepare a sufficient number of microtubes to accommodate samples and controls.

1. Pipette **20 µl** of LAMP reaction buffer into the tubes.
2. Incubate for 5 minutes at room temperature (18 - 28°C).
3. For each test vial add **2 µl** sample (isolated RNA) or positive- or negative controls.
4. Close reaction tubes
5. Mix until dried reagent mixture is completely dissolved
6. Incubate for **45 min at 65 °C.**
7. Cool for 5 minutes at room temperature
8. Evaluate samples, the positive control must be blue-green and the negative control pink. Read individual samples according the negative and positive control.

6.6 INTERPRETATION OF RESULTS

Possible results of the different SARS-COV-2 Detection Assays:

Marker	British Lineage B1.1.7	South African B.1.351	Brazilian P1	SARS-COV2 Hu-1
N501Y	+	+	+	-
HV69/70 Deletion	+	-	-	-
E484K	-	+	+	-
K417T	-	-	+	-
E1/O117 gene	+	+	+	+

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7 FLOWCHART

Pipette **20 µl** LAMP reaction buffer into each reaction tube

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Incubate for **5 minutes** at room temperature

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Add **2 µl** RNA sample or control

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Close reaction tubes

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Mix until dried reagent mixture is completely dissolved

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Incubate for **45 minutes** at **65 °C** temperature

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Cool for **5 minutes** at room temperature

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Read color for negative reaction (slight pink or positive reaction (blue-green)

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8 LITERATURE

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