DeepChek

Whole Genome SARS-CoV-2 Genotyping (RUO)

V1

User Guide

48

Version 1 – Revision 1

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

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Application

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The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** is a reverse transcriptase (RT) polymerase chain reaction (PCR) test (nucleic acid technique (NAT)) intended to screen the emergence of SARS-CoV-2 genome mutations using extracted RNA from patients already diagnosed PCR positive to SARS-CoV-2.

The test is amplifying the whole genome of the SARS-CoV-2, including regions which harbor mutations described as sufficient, when present, to characterize some variants.

Results are used to build basic research knowledge and evidence on the emergence of circulating SARS-CoV-2 variants from lineages B.1.1.7 (United Kingdom, i.e. VOC 202012/01, VOC 202102/02), B.1.351 (South Africa, i.e. VOC 202012/02) or P.1 (derived from B.1.1.28, Brazil, i.e. VOC 202101/02).

Results are used to better understand SARS-CoV-2 transmission and to monitor for the emergence of new variants, even in minor viral populations, through ongoing epidemiological surveillance and strategic testing; conducting outbreak investigation and contact tracing; and where appropriate, adjusting public health and social measures to reduce transmission of SARS-CoV-2.

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of RT-PCR and next generation sequencing (NGS) workflow.

Principles of the assay

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** is a reverse transcription polymerase chain reaction multiplex test which includes ninety-eight pairs of primers, reverse and forward, designed to detect SARS-CoV-2 RNA from individuals already tested SARS-CoV-2 positive.

During the amplification reaction, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable DNA polymerase. First, the SARS-CoV-2 reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of the 98 targets takes place simultaneously in the same thermal cycling program in two distinct wells.

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** is performed on a PCR instrument.

Subsequently, the amplicons can be used for next generation sequencing and analysed with a downstream analysis software to list SARS-CoV-2 mutations and issue a report with a list of mutations which can be filtered with available public reference knowledge.

Assay components

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** is provided in one model of 48 reactions (REF 159A48). The specified number of reactions is the number of reactions for each target.

Label	Volume for 48 Rn.	Color cap	Storage
Master Mix 2X	1 * 1320 µL	Green	-25°C to - 15 °C
RT Mix	1 * 26 µL	Pink	-25°C to - 15 °C
WG Primers Pool#1	1 * 190 µL	Yellow	-25°C to - 15 °C
WG Primers Pool#2	1 * 190 µL	Yellow	-25°C to - 15 °C
Water	1 * 280 µL	Blue	-25°C to - 15 °C
Positive control	1 * 20 µL	Clear	-25°C to - 15 °C
Negative control	1 * 20 µL	Black	-25°C to - 15 °C

*Table 1 : Volumes and storage conditions of the **DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1.1 (RUO)***

Reagent storage and handling

The ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** should be stored at - 25°C to - 15 °C and is stable until the expiration date stated on the label. Note the production date and expiration date listed on the label. Reagents from different lot numbers should not be mixed.

Note: Multiple thaw-freeze cycles should be avoided. Aliquoting should be considered.

Materials required but not provided

- Any validated instrument for RNA extraction and purification using magnetic-bead technology.
- PCR instrument i.e. ThermoFisher Scientific Proflex PCR System and associated specific material or any thermal cycler with enough ramp rate of ≥ 1°C/s.
- Benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
- Benchtop vortex mixer.
- Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Pipetting Robot (optional).
- Reagents for 0.8–2% agarose gel in 0.5x TBE electrophoresis buffer or equivalent capillary electrophoresis reagent, i.e. Agilent ScreenTape D1000 and Reagents D1000 for Agilent TapeStation 4150.
- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters.
- Adjustable pipettes & fitting filtered pipette tips.
- Appropriate PPE & workspaces for working with potentially infectious samples.

- Surface decontaminants such as DNAZap (Life Technologies), DNA Away (Thermo Fisher Scientific), RNAse Away (Thermo Fisher Scientific), 10% bleach.
- Nuclease-free dH2O.
- 0.5 ml or 1.5 ml RNase- and DNase-free PCR tubes.
- Ice/Icebox or even cooling blocks.
- 96 well plate cooler (optional).
- 96 well PCR plates.
- Plate thermo seals.
- Plate centrifuge.
- 0.2 mL thin walled 8 tube & domed cap.

Note:

- Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.
- Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

Warnings and precautions

- **For Research Use Only (RUO). Not for use in diagnostic procedures.** No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.
- This product has been tested only for nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- Handle all specimens as of infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafetyguidelines.html
- Laboratories maybe required to report all test results to the appropriate public health authorities.
- Store assay reagents as indicated on their individual labels.
- Do not mix reagents from different kit lots
- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- Work surfaces and pipettes should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- When a positive control is used, it should be handled in an area separate from sample receiving, accessioning and processing areas to avoid contamination of the samples with amplifiable material.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Frequent cleaning of the wells of the PCR instrument plate is recommended to prevent contamination.
- To avoid contamination, use separated and segregated working areas: 1) Reagent preparation area – preparing the reagents for amplification, 2) Dilution of positive control material according laboratory guidelines, 3) sample preparation area- isolation of the RNA/ DNA from sample and control, and 4) Amplification area- amplification and detection of nucleic acid targets.

- Check whether the PCR reaction tubes are tightly closed before loading on the PCR instrument to prevent contamination of the instrument from leaking tubes.

Workflow

1. Batches of clinical samples tested for SARS-CoV-2.
    - Negative and positive results are given to healthcare provider
    - PCR positive results are kept for second testing
2. Extracted RNA from positive results of previous PCR runs are directly used with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO):***
    a) PCR master mix preparation
    b) PCR reaction setup
    c) PCR run
    d) Quality control and analysis of the amplicon
    e) Next generation sequencing (NGS)
    f) Analysis of NGS raw data
    g) Laboratories reporting to the appropriate public health authorities.

- It is recommended to use extracted RNA from positive results of previous PCR run with a Ct < 28.
- Pool RNA quality might result in failure to amplify the targets, a new RNA isolation should be considered.
- If the downstream PCR and library preparation reactions of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** are not carried out immediately after the SARS-CoV-2 detection PCR run, extracted nucleic acid should be stored at -70°C or lower.
- It is recommended to make few aliquots of RNA extracts to prevent thawing and unthawing.

- Do not vortex specimens as this will fragment the RNA and lead to failure of the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** tests.

Starting

- Identify the product.
- Verify the expiration date.
- Verify the latest instruction for use available for the product lot number.
- Verify if the product was used already. If yes, check the remaining tests available.

Starting material for the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** is extracted RNA from SARS-CoV-2 positive PCR.

PCR reaction setup

Note: Plate set-up configuration can vary with the number of specimens and workday organization.

1. Thaw the following reagents on ice: Master Mix 2x; RT Mix, WG Primers Pool#1, WG Primers Pool#2 and water.
2. Briefly centrifuge (2000rpm, 10sec) the reagents to collect the contents.
3. Prepare the 2 master mixes, "Pool1" and "Pool2", according to the tables below. The master mix typically contains all the components required for RT-PCR except the template RNA. Prepare a volume for each master mix greater (n+1) than that required for the total number of reactions to be performed. The master mix shall be kept on ice.

Reagent	Volume / Reaction	
	Pool #1	Pool #2
Master Mix 2X	12.50 µL	12.50 µL
RT Mix	0.25 µL	0.25 µL
WG Primers Pool# *n*	3.60 µL	3.60 µL
Water	2.65 µL	2.65 µL
Total Volume	19.00 µL	19.00 µL

Table 2 : Master mix preparation for each Pool

4. Add **6 µL** of extracted RNA solution to a single PCR tube.
5. Do not add more than one sample of extracted RNA into a single qPCR tube. **Each PCR tube shall have a total volume of 25 µL. Then immediately close the tubes and transfer the reaction setup into a PCR machine for the amplification.**

Note : Prepare a volume of master mix greater (n+2) if you use a Positive Control.

Quality controls

Controls that are provided with the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** are listed below:

a) A "no template" (negative) control (NTC) consisting of Water (molecular grade) is used and is needed to detect cross-contamination during all reaction steps. The NTC is used during extraction and PCR and is used to determine validity of the test run.
b) A positive template control [(ZeptoMetrix SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) (Catalog# 0810587CFHI)] is needed to ensure the RT-PCR reaction setup and reagent integrity and is used to validate the RT-PCR plate results. The positive control is used during PCR.

PCR cycling condition

Set up the following thermal cycling program.

Stage	Cycle	Ramping rate (°C/s)	Temperature (°C)	Time (min:sec)
1	1	2.0	50	10:00
2	1	2.0	95	5:00
3	1	2.0	98	00:30
4	35	2.0	95	00:15
		2.0	63	05:00
5	1	2.0	4	HOLD

Table 3: PCR cycling program

Sample quality control

Sample results are only valid when negative controls yield no amplification. If the NTC is invalid in an assay, all sample results in the same assay are therefore also invalid and the operator shall repeat testing of the entire batch. All test controls should be examined prior to interpretation of patient results. If any of the controls are not valid, the patient results cannot be interpreted.

PCR products can be controlled through electrophoresis on an agarose gel (2%). Check the intensity of the signal. Even if low-intensity bands usually leads to a successful sequencing, it is recommended to avoid the process if no band can be observed.

The expected amplicons size of the fragment, both for Pool#1 and Pool#2, shall be approximatively of 400 bp with 2% of agarose.

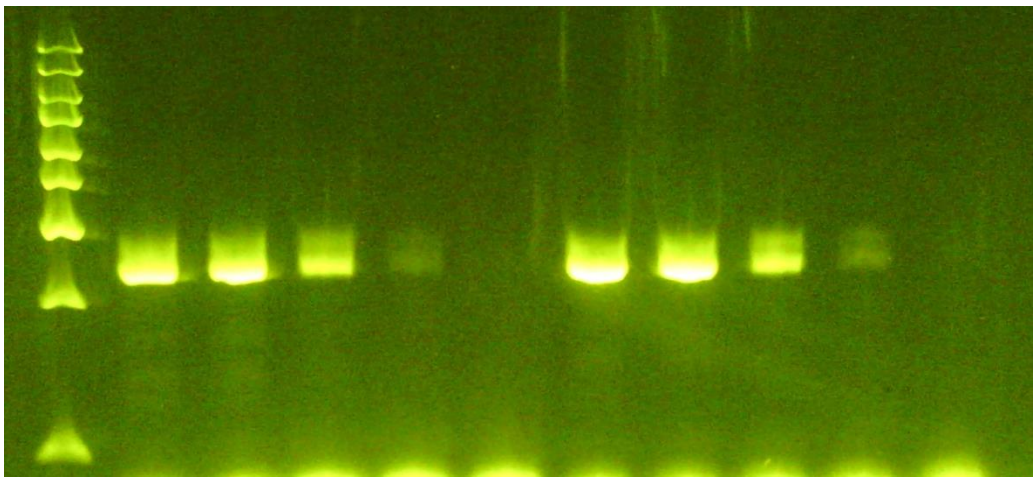

Figure 1 : Example of an agarose gel (2%) of a successful PCR for Pool#1 and Pool#2 Whole Genome

Post PCR

Next Generation Sequencing (NGS)

After the amplicon verification, the samples are ready for the NGS workflow processing:

Through Illumina (MiSeq / iSeq 100)

- **116AX** | DeepChek® NGS Library preparation (24 or 48 or 96 tests)
- **124AX** | DeepChek® Assay Adapters (1-24, 1-48 or 1-96)
- **MS-103-1003** | MiSeq Reagent Nano Kit, v2 (500 cycles)
- **MS-102-3003**| MiSeq Reagent Kit, v3 (600 cycles)
- **20021532** | iSeq 100 Sequencing System
- **20021533** | iSeq 100 Reagent (300 cycles)

Note: If you are using MiSeq Reagent Nano Kit, v2 (500 cycles) or MiSeq Reagent Kit, v3 (600 cycles), no need to perform the fragmentation step with the DeepChek® NGS Library preparation (**116AX**).

Through Thermo Fisher Scientific (Ion Torrent)

- **4471269** | Ion Xpress™ Plus Fragment Library Kit
- **4471250** | Ion Xpress™ Barcode Adapters 1-16 Kit
- **4484355** | Ion 318™ Chip Kit v2

Downstream NGS Analysis Software

The next generation sequencing raw data are uploaded in a specific downstream software tailored for SARS-CoV-2 analysis which is a standalone medical device (i.e. ***DeepChek CoV2 software (RUO)*** licence and module (REF S-12-023 (CVL) and S-12-023 (CVM)). The software itself could be CE-IVD marked and shall refer to updated expert list of mutations and variants per virus lineages with latest knowledge and analyses.

List of mutations covered by the Assay

As of today, the listed mutations hereafter shall be present to characterize the variants of concern.

Mutations covered by the ***DeepChek Assay Whole Genome SARS-CoV-2 Genotyping V1 (RUO)*** are in bold underlined text and could be reported by the ***DeepChek CoV2 software (RUO)*** or any other standalone medical device intented by the manufacturer to analyze SARS-CoV-2 next generation sequencing raw data.

- Variant B.1.1.7 [United Kingdom] : substitutions **N501Y, A570D, P681H, T716I, S982A, D1118H** and **deletions del69-70 (HV), del144/145 (Y)**;
- Variant B.1.1.7 [United Kingdom cluster with E484K] : substitutions **E484K, N501Y, A570D, P681H, T716I, S982A, D1118H, L730F, A173V, A398T** and **deletions del69-70 (HV), del144/145 (Y)**;
- Variant B.1.351 [South Africa] : substitutions **D80A, D215G, E484K, N501Y, A701V** potentially associated to substitutions **L18F, R246I, K417N and deletion del L242-244L or substitution L242H**;
- Variant P.1 (derived from B.1.1.28) [Brazil] : **L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I.**

Limitations

- **For Research Use Only (RUO). Not for use in diagnostic procedures.** No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

- Laboratories maybe required to report all test results to the appropriate public health authorities.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may affect the test performance.
- A false negative result may occur if a specimen is improperly stored or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the test target regions, SARS-CoV-2 RNA may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- False-positive results may arise from cross contamination during specimen handling, preparation, assay set-up or product handling.
- The lack of a mutation on a Variant Calling Report issued by a downstream next generation sequencing raw data analysis software does not preclude the possibility of genetic mutation.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic, immunosuppressant drugs or cold medications have not been evaluated.

Product quality control

In accordance with ABL's Quality Management System, each lot of the assay is tested against predetermined specifications to ensure consistent product quality. Certificates of Analysis are available upon request.

Symbols

Σ <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution	CONTROL -	Negative control
REF	Catalog number	CONTROL +	Positive control
	Use by		Temperature limitation
	Manufacturer	SN	Serial Number
	Country of manufacturing	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Distributor		

Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: https://ablsa.odoo.com/fr_FR/issue; Email: support-diag@ablsa.com; Call +339 7017 0300 Or contact your ABL Field-Application Specialist or your local distributor. For up to date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide. ABL User Guides are available at **www.ablsa.com/ifu** or can be requested from ABL Technical Services or your local distributor.

Manufacturer

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The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument. The information in this guide is subject to change without notice. DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, ABL (S.A) AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Version 1.1

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