DeepChek® Assay

UL54 / UL56 / UL97 Drug Resistance

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User Guide

Version 1 – Revision 0

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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Document control

Date	Device version	IFU version	Description of change
2022/12/29	B	1.0	New version of the assay: changes in assay components and in the protocol (new region UL56)
2022/07/01	A	1.0	Document creation

Contents

Application

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The ***DeepChek® Assay UL54 / UL56 / UL97 Drug Resistance (RUO)*** is a polymerase chain reaction (PCR) test (nucleic acid technique (NAT)) intended to screen mutations in the cytomegalovirus (CMV) genes, UL54, UL56 and UL97, which are associated with antiviral resistance.

The ***DeepChek® Assay UL54 / UL56 / UL97 Drug Resistance (RUO)*** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of PCR and next generation sequencing (NGS) workflow.

Principles of the assay

The ***DeepChek® Assay UL54 / UL56 / UL97 Drug Resistance (RUO)*** is a polymerase chain reaction test which includes primers, reverse and forward, designed to amplify CMV DNA from extracted specimens.

After the initial denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded DNA, CMV forward and reverse primers anneal to the DNA strand and is extended by the DNA polymerase activity of the enzyme to create a double-stranded DNA product of the target gene.

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 targets takes place simultaneously in the same thermal cycling program in three (3) distinct wells.

The ***DeepChek Assay UL54 / UL56 / UL97 Drug Resistance (RUO)*** is performed on a PCR instrument.

Subsequently, the amplicons can be used for next generation sequencing and analyzed with a downstream analysis software to list in a report detected CMV genome mutations according to available public reference knowledge databases.

Genotypic analysis of various regions of CMV facilitates the study of the relationship between mutations and antiviral resistance.

Assay components

The ***DeepChek® Assay UL54 / UL56 / UL97 Drug Resistance V2 (RUO)*** is provided in one model of 24 reactions (REF 117B24).

Table 1: Volumes and storage conditions of the ***DeepChek® UL54 / UL56 / UL97 Drug Resistance V2 (RUO)***

Label	Volume for 24 Rxn (nb tube x volume)	Color cap	Storage
Master Mix	5 x 3700 µL	Green	-25°C to - 15 °C
DMSO	1 x 115 µL	Pink	-25°C to - 15 °C
UL54 FOR Primers (50 µM)	1 x 15 µL	Yellow	-25°C to - 15 °C
UL54 REV Primers (50 µM)	1 x 15 µL	Purple	-25°C to - 15 °C
CMV_UL56_F5 (50 µM)	1 x 15 µL	Orange	-25°C to - 15 °C
CMV_UL56_R5 (50 µM)	1 x 15 µL	Black	-25°C to - 15 °C
UL97 FOR Primers (50 µM)	1 x 15 µL	Red	-25°C to - 15 °C
UL97 REV Primers (50 µM)	1 x 15 µL	Brown	-25°C to - 15 °C
H_2O	1 x 500 µL	Blue	-25°C to - 15 °C

Master Mix	Master Mix	Master Mix	Master Mix	Master Mix
DMSO	H_2O		UL54 FOR Primers	UL54 REV Primers
UL97 FOR Primers	UL97 REV Primers		CMV_UL56_F5	CMV_UL56_R5

*Figure 1: Disposal of the assay components for the **DeepChek® UL54 / UL56 / UL97 Drug Resistance V2 (RUO)***

Reagent storage and handling

The ***DeepChek® Assay UL54 / UL56 / UL97 Drug Resistance (RUO)*** is shipped with dry ice and should be maintained and stored immediately upon receipt at –20°C to avoid compromising cold chain integrity.

Expiration date: please refer to the label on the kit box.

Materials required but not provided

- Any validated instrument for DNA extraction and purification using magnetic-bead technology.
- PCR instrument e.g., 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, e.g., 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 the amplification of nucleic acid from cytomegalovirus (CMV), not for any other viruses or pathogens.
- Handle all specimens as of infectious using safe laboratory procedures.
- 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 the 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.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious specimens.
- 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.
- Check whether the PCR reaction tubes are tightly closed before loading on the PCR instrument to prevent contamination of the instrument from leaking tubes.

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.

DNA Extraction

To achieve optimal and sensitive CMV DNA analysis, the best representation of the viral quasispecies, it is recommended to extract **one (1) or two (2) mL** of specimen (e.g., plasma, whole blood …) for subsequent DNA and amplicon generation and to be eluted in the minimum volume required for your preferred extraction kit.

Note: MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics) was tested efficiently.

The ***DeepChek® Assay UL54 / UL56 / UL97 Drug Resistance*** will work with at least an extraction of 1 mL of specimen, ideally fresh, to be eluted in 100 µL.

Note**:** sensitivity was evaluated on few specimens around 1250 UI/mL.

Note**:** for specimens with low viral load, we recommend performing an ultracentrifugation procedure. Pellet the virus for 1.5 hours at 40,000 g (or alternatively for 2 hours at 24,000g), and at 4°C. Remove enough supernatant to leave the required amount of specimen for your preferred extraction kit.

PCR set-up

1. Thaw extracted template DNA, primer solutions, Master Mix, RNase-free water, DMSO and place them on ice. Load all the tubes into the centrifuge. Spin the samples at 11000g for 10 seconds. And then aspirate and discharge the solution several times before the dispensing.
2. Prepare a master mix according to the next table. The master mix typically contains all the components required for PCR except the template DNA. Prepare a volume of master mix greater (n+1) than that required for the total number of reactions to be performed.

Table 2: Reaction components for UL54 and UL97 targets

Reagent	UL54 Volume	UL56 Volume	UL97 Volume
Master Mix	25.0 µL	25.0 µL	25.0 µL
DMSO	1.5 µL	1.5 µL	1.5 µL
UL54 FOR Primers (50 µM)	0.4 µL	-	-
UL54 REV Primers (50 µM)	0.4 µL	-	-
CMV_UL56_F5 (50 µM)	-	0.4 µL	-
CMV_UL56_R5 (50 µM)	-	0.4 µL	-
UL97 FOR Primers (50 µM)	-	-	0.4 µL
UL97 REV Primers (50 µM)	-	-	0.4 µL
H_2O	2.7 µL	2.7 µL	2.7 µL
Total volume / sample	**30.0 µl**	**30.0 µl**	**30.0 µL**

3. Vortex the master mix thoroughly and dispense 30 µL into PCR tubes. Mix by pipetting the master mix up and down a few times.
4. Add 20 µL of DNA to the PCR tubes. Mix by pipetting the master mix up and down a few times.
5. Program the thermal cycler according to the following program.

Table 3: PCR UL54, UL56 and UL97 Cycling Program

Cycle	Temperature (°C)	Time
Enzyme activation	94	30 sec
45 cycles	94	35 sec
	60	30 sec
	65	4 min 15 sec
Final extension	65°C	10 min
	10	∞

6. Start cycling program while PCR tubes are still on ice. After amplification, samples can be stored overnight at 2–10°C or at –20°C for long-term storage.
7. [Recommended] - PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of the signal. Even if low-intensity bands usually lead to a successful sequencing, it is recommended to avoid the process if no band can be observed.

Note**:** the expected amplicons size after the PCR step are:

- **UL54: ~4000 bp**
- **UL56: ~2500 bp**
- **UL97: ~2000 bp**

PCR troubleshooting guide

1. Check the concentration, storage conditions and quality of the starting template. For optimal results use fresh/frozen specimen and proceed with a fresh DNA extraction.
2. For specimens with low viral load, we recommend performing an ultracentrifugation procedure. Pellet the specimen for 1.5 hours at 40,000 g and at 4°C. Remove enough supernatant to leave the required amount of specimen for your preferred extraction kit.

PCR products purification

Before sequencing, first make sure your PCR products have been purified.

Note**:** for amplicons greater than 500 bp, use 0.6 * DeepChek® NGS Clean-up beads (REF N411-02) (30 µl volume of beads). For amplicons smaller than 500 bp, use 1.8 * DeepChek® NGS Clean-up beads (90 µl volume of beads) to maximize yield.

Next Generation Sequencing

After the amplicon verification, the specimens are ready for the NGS kit processing, with Illumina:

- **116B24 / 116B48 / 116B96 | ABL DeepChek® NGS LIBRARY PREPARATION V2 (24/48/96 reactions).**
- **124B24 / 124B48 / 124B96|ABL DeepChek® Adapters V2 (24 / 48 / 96).**
- **MS-103-1003 |** MiSeq Reagent Nano Kit, v2 (500 cycles) or
- **FC-420-1003 |** Mid Output kit Reagents (2x150) or
- **20021533 |** iSeq 100 i1 Reagent (2x150) or
- **20024908 |** NextSeq 500/550 High Output Kit v2.5 (300 Cycles).

User shall then follow the Denature and Dilute Libraries Guide and instructions for use from the manufacturer.

Through Ion Torrent: **4471269** | Ion Xpress™ Plus Fragment Library Kit, **4471250** | Ion Xpress™ Barcode Adapters 1-16 Kit and **4484355** | Ion 318™ Chip Kit v2. User shall then follow the instructions for use from the manufacturer.

NGS data analysis

NGS files containing nucleotide sequences for the specimen are analyzed by a downstream analysis software (e.g., the ABL **DeepChek® Software** with the CMV module (REF S-12-023 (MM)). Users shall then follow the software user guide.

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

 <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution		Temperature limitation
	Catalog number		Serial Number
	Use by	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Manufacturer		Distributor
	Country and date of manufacturing		

Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: support-diag.ablsa.com; 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 and distributors

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