DeepChek® Assay

RT Genotyping and Drug Resistance (RUO)

V1

User Guide

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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.

REF **113A24 (old reference: K-17-B-RT)**

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Document control

Date	Device version	IFU version	Description of change
28/03/2022	A	1.1	• Change of the analytical sensitivity to 1000 UI/mL in RNA extraction section • Update the list of next generation sequencing instruments and reagents

Contents

Application

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The DeepChek® Assay RT genotyping and Drug Resistance (DR) (RUO) kit is a single tube system which utilizes PCR technology for amplifying relevant portions of the human B virus (HBV) RT gene from input DNA extracted from plasma/serum.

This nucleic acid amplification method might aid in the typing of HBV viruses and DR. This test is NOT intended to be used as a screening or confirmation test for the detection, confirmation, and quantification of HBV infection.

The DeepChek® Assay RT genotyping and Drug Resistance (RUO) is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of PCR, SANGER and next generation sequencing (NGS) workflow.

Principles of the assay

The DeepChek® Assay RT genotyping and Drug Resistance (RUO) is a reverse transcriptase-polymerase chain reaction test which includes primers, reverse and forward, designed to amplify HBV DNA from plasma/serum specimens.

First, the HBV 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 DNA, a second primer anneals to the DNA 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.

DeepChek® Assay RT genotyping and Drug Resistance (RUO) is performed on a PCR instrument.

Subsequently, the amplicons can be used for SANGER or next generation sequencing and analyzed with a downstream analysis software to list in a report HBV genotypes according to available public reference knowledge databases.

Assay components

The ***DeepChek® Assay RT V1 (RUO)*** is provided in one model of 24 reactions (REF 113A24 / OLD REF K-17-B-RT).

Label	Volume for 24 Rxn	Color cap	Storage
Master Mix 2X	1 x 800 µL	Green	-25°C to - 15 °C
G Frag HBV RT-FOR Primers	1 x 40 µL	Yellow	-25°C to - 15 °C
H Frag HBV RT-FOR Primers	1 x 40 µL	Yellow	-25°C to - 15 °C
Sequencing G Frag HBV RT-FOR Primers	1 x 32 µL	Red	-25°C to - 15 °C
Sequencing H Frag HBV RT-FOR Primers	1 x 32 µL	Red	-25°C to - 15 °C
H_2O	1 x 500 µL	Blue	-25°C to - 15 °C
G Frag HBV RT-REV Primers	1 x 40 µL	Yellow	-25°C to - 15 °C
H Frag HBV RT-REV Primers	1 x 40 µL	Yellow	-25°C to - 15 °C
Sequencing G Frag HBV RT-REV Primers	1 x 32 µL	Red	-25°C to - 15 °C
Sequencing H Frag HBV RT-REV Primers	1 x 32 µL	Red	-25°C to - 15 °C

*Table 1: Volumes and storage conditions of the **DeepChek® Assay RT Genotyping and Drug Resistance V1 (RUO)***

Reagent storage and handling

The ***DeepChek® Assay RT V1 (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

- Thermocycler ABI9700
- 96-well plate cooler (Eppendorf / 22510509)
- 96-well PCR plates (Eppendorf / 951020303)
- Plates thermo seals (Thermo Scientific / AB-0558)
- Plate centrifuge
- 0.2 mL thin-wall 8 tubes & domed caps (Thermo Scientific / AB-0266)
- 1.5 mL centrifuge tubes (Dot Scientific Inc. / RN1700-GST)
- Centrifuges tubes (see your specific centrifuge manual)
- Mini centrifuge (see your specific centrifuge manual)
- Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL)
- Ice

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.
- 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.
- 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.
- 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.

RNA Extraction

To achieve optimal and sensitive HBV DNA analysis, the best representation of the viral quasispecies, it is recommended to extract **1 mL** of plasma for amplicon generation and elute in the minimum volume required for your preferred extraction kit.

The ***DeepChek® Assay RT V1 (RUO)*** will work with at least an extraction of 400 µL of plasma or serum, ideally from fresh samples, to be eluted in 100µL (related sensitivity evaluated to 1000 UI/mL).

For samples with low viral load, we recommend:

1. To perform 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 sample for your preferred extraction kit.

OR

2. To extract one or 2 mL of plasma/serum and elute in the minimum volume required for your preferred extraction kit.

PCR Step-by-Step Workflow

1. Thaw extracted template DNA, primer solutions, Master Mix and place them on ice. Load all the tubes into the centrifuge. Spin the samples at 10000 RPM for 10 seconds. And then aspirate and discharge the solution several times before the dispensing.
2. Prepare G fragment and H fragment master mix according to **Table 2**. G and H master mix must be prepared separately. 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.

PCR Reagent	G Frag Volume	H Frag Volume
Master Mix 2X	12.5 µL	12.5 µL
G Frag-HBV-RT-FOR Primers 10 µM	1.25 µl	-
G Frag-HBV-RT-REV Primers 10 µM	1.25 µL	-
H Frag-HBV-RT-FOR Primers 10 µM	-	1.25 µL
H Frag-HBV-RT-REV Primers 10 µM	-	1.25 µL

Table 2: Reaction components for the G and H fragments PCR target

3. Vortex the master mix thoroughly and dispense 15 µL into PCR tubes. Mix by pipetting the master mix up and down a few times.
4. Add 10µL of DNA in the PCR tubes. Mix by pipetting the master mix up and down a few times.

5. Program the thermal cycler according to the program in **Table 3**.

Cycle	Temperature (°C)	Time
Enzyme activation	95	5 min
10 cycles	95	15 sec
	68	30 sec
	72	1 min
35 cycles	95	15 sec
	68	30 sec
	72	2 min
Final extension	72	10 min
1	10	∞

Table 3: G and H fragments PCR Cycling Program

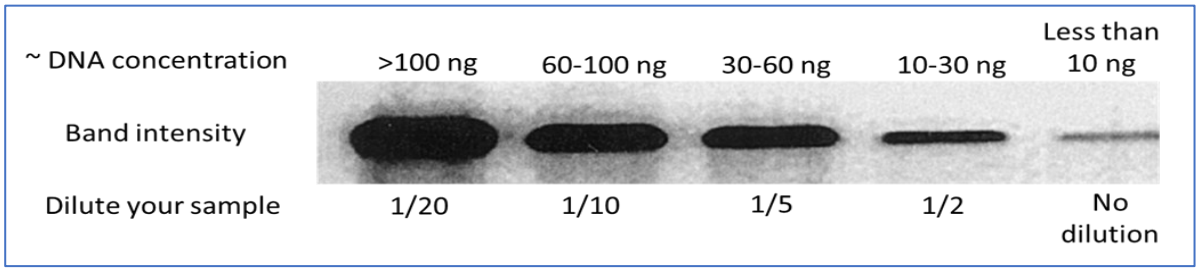
6. Start the DeepChek® Assay RT cycling program while PCR tubes are still on ice. After amplification, samples can be stored overnight at 2–10°C, or at –20°C for long-term storage.
7. **[Recommended]** - PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of 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.

Expected amplicons size:

- **G Fragment: 1563 bp**
- **H Fragment: 760 bp**

RT-PCR Troubleshooting Guide

1. Check the concentration, storage conditions and quality of the starting template. For optimal results use fresh/frozen plasma/serum and proceed with fresh RNA extraction.
2. For samples with low viral load, we recommend performing an ultracentrifugation procedure. Pellet the virus for 1.5 hours at 40,000 g and at 4°C. Remove enough supernatant to leave the required amount of sample for your preferred extraction kit.
3. In presence of very large PCR bands on the agarose gel, dilute ($1/10^1$ - $1/10^3$) of the product before sequencing.



PCR Products Purification

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

Sequencing

1. Purification

For amplicons greater than 500bp, use a 0.6x AMPure XP cleanup (30 µl volume of beads). For amplicons less than 500bp, use a 1.8x AMPure XP cleanup (90 µl volume of beads) to maximize yield.

2. Sanger sequencing

In presence of very large PCR bands on the agarose gel, make dilution ($1/10^1$ - $1/10^3$) of the SingleRound RT-PCR product before sequencing.

For G fragment sequencing use the 2 red cap tubes (each well containing 29µL of each Sanger sequencing primer (forward and reverse - 3.2 µM). **1µL** of each primer will be used for each sample and diluted in a final volume of 10 µl (the final concentration will be 0.32µM / primer /sample).

For H fragment sequencing use the 2 red cap tubes (each well containing 29 µL of each Sanger sequencing primer (forward and reverse - 3.2 µM). **1µL** of each primer will be used for each sample and diluted in a final volume of 10 µl (the final concentration will be 0.32µM / primer /sample).

1. Prepare the sequencing reaction according to the **Table 4a** (Big Dyes Terminator kit v1.1) or **4b** (Big Dyes Terminator kit v3.1).

Reagent	Volume
Big Dye Terminator v1.1	1 µL
Sequencing Buffer	1 µL
Primer (3,2µM)	**1 µL**
Purified RT-PCR	0.7 – 2 µL
Water	q.s. to 10 µL

Table 4a: Sequencing reaction for Big Dyes Terminator kit v1.1

Reagent	Volume
Big Dye Terminator v3.1	2 µL
Sequencing Buffer (5X)	1 µL
Primer (3,2µM)	**1 µL**
Purified RT-PCR	0.7 – 2 µL
Water	q.s. to 15 µL

Table 4b: Sequencing reaction for Big Dyes Terminator kit v3.1

2. Program the thermal cycler according to the program in **Table 5a** (Big Dyes Terminator kit v1.1) or **5b** (Big Dyes Terminator kit v3.1).

Cycle	Temperature (°C)	Time
1	96	5 min
25 cycles	96 50 60	10 sec 5 sec 4 min
1	4	∞

Table 5a: Thermal cycler for Big Dyes Terminator kit v1.1

Cycle	Temperature (°C)	Time
1	96	1 min
25 cycles	96 50 60	10 sec 5 sec 4 min
1	4	∞

Table 5b: Thermal cycler for Big Dyes Terminator kit v3.1

3. Sephadex Complete all the sequencing reaction with 10µL of water (q.s. to 20µL).
4. Purify all sequencing reaction (20µL) with Sephadex gel before the final Sanger sequencing.

3. Next Generation Sequencing

The main volume of the product output is then used for next generation sequencing (NGS). The NGS workflow can be different as the NGS technics and NGS analyzers vary. NGS sequencing instruments are general laboratory use devices. Sequencing and library preparations reagents are general laboratory use products.

After the amplicon verification, the samples are ready for the NGS kit processing.

Through Illumina (MiSeq / iSeq 100)

- **116BX** | DeepChek® NGS Library preparation V2 (24 or 48 or 96 tests)
- **124BX** | DeepChek® Assay Adapters V2 (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

Data Analysis

1. Sanger

AB1 or FASTA files containing nucleotide sequences for G and H fragments are analyzed by the **DeepChek®** software. User shall then follow the DeepChek® software procedure to complete the data analysis and reporting processes.

2. NGS

NGS files containing nucleotide sequences for G and H fragments are analyzed by the **DeepChek®** software. User shall then follow the DeepChek® software procedure to complete the data analysis and reporting processes.

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		Negative control
	Catalog number		Positive control
	Use by		Temperature limitation
	Manufacturer		Serial Number
	Country of manufacture with a date of manufacture	**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

Advanced Biological Laboratories (ABL) S.A.

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Version 1.1

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