DeepChek® Assay

Whole Genome HBV Genotyping

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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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Contents

Application

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The ***DeepChek® Assay Whole Genome HBV Genotyping*** is a polymerase chain reaction (PCR) test (nucleic acid technique (NAT)) intended to screen the HBV mutations and HBV genotypes.

The test is amplifying, the whole genome of the Hepatite B virus in HBV specimens, including regions which harbor mutations described as sufficient, when present, to determine level of resistance to anti-retroviral drugs.

The ***DeepChek® Assay Whole Genome HBV Genotyping*** 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 Whole Genome HBV Genotyping*** *is* a polymerase chain reaction test which includes primers, reverse and forward, designed to amplify HBV-DNA from extracted specimens. The various sets are available in three (3) distinct wells.

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 Whole Genome HBV Genotyping*** 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 HBV genome mutations according to available public reference knowledge databases.

Genotypic analysis of various regions of HBV facilitates the study of the relationship between mutations and viral resistance to anti-retroviral drugs, specifically the polymerase, the pre-coce, the core, the pre S1/2 and ORF S.

Assay components

The ***DeepChek® Assay Whole Genome HBV Genotyping*** is provided in a single model of 24 reactions (REF 184A24).

Table 1: Volumes and storage conditions of the DeepChek Assay Whole Genome HBV Genotyping

Label	Volume for 24 Rxn. (nb. tube x volume)	Color cap	Storage
Master Mix 2X	1 x 1000 µL	Green	-25°C to -15°C
Frag 1 (20 µM)	1 x 110 µL	Yellow	-25°C to -15°C
Frag 2 (20 µM)	1 x 110 µL	Orange	-25°C to -15°C
Frag 3 (20 µM)	1 x 110 µL	Brown	-25°C to -15°C
Nested Master Mix 2X	1 x 1000 µL	Green	-25°C to -15°C
Nested Frag 1 (20 µM)	1 x 110 µL	Pink	-25°C to -15°C
Nested Frag 2 (20 µM)	1 x 110 µL	Purple	-25°C to -15°C
Nested Frag 3 (20 µM)	1 x 110 µL	Red	-25°C to -15°C
H_2O	1 x 500 µL	Blue	-25°C to -15°C

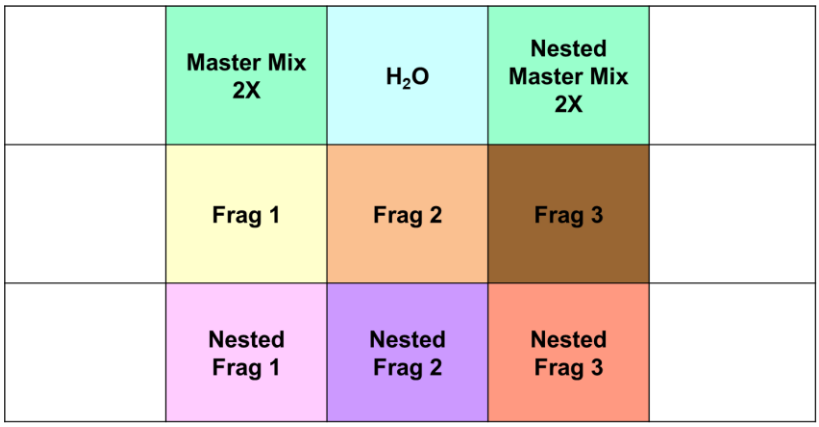

Figure 1: Disposal of the assay components for the DeepChek® Assay Whole Genome HBV Genotyping

Reagent storage and handling

The ***DeepChek® Assay Whole Genome HBV Genotyping*** 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 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 HBV, 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 HBV DNA analysis for subsequent amplicon generation, and downstream next generation sequencing, when using the ***DeepChek® Assay Whole Genome HBV Genotyping***, it is recommended to work with at least an extraction of 1 mL of specimen (e.g., plasma, serum) to be eluted in 50 µL.

For specimens 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 specimen for your preferred extraction kit.
   OR
2. To extract 1-2 mL of specimen and elute it in the minimum volume required for your preferred extraction kit.

Workflow

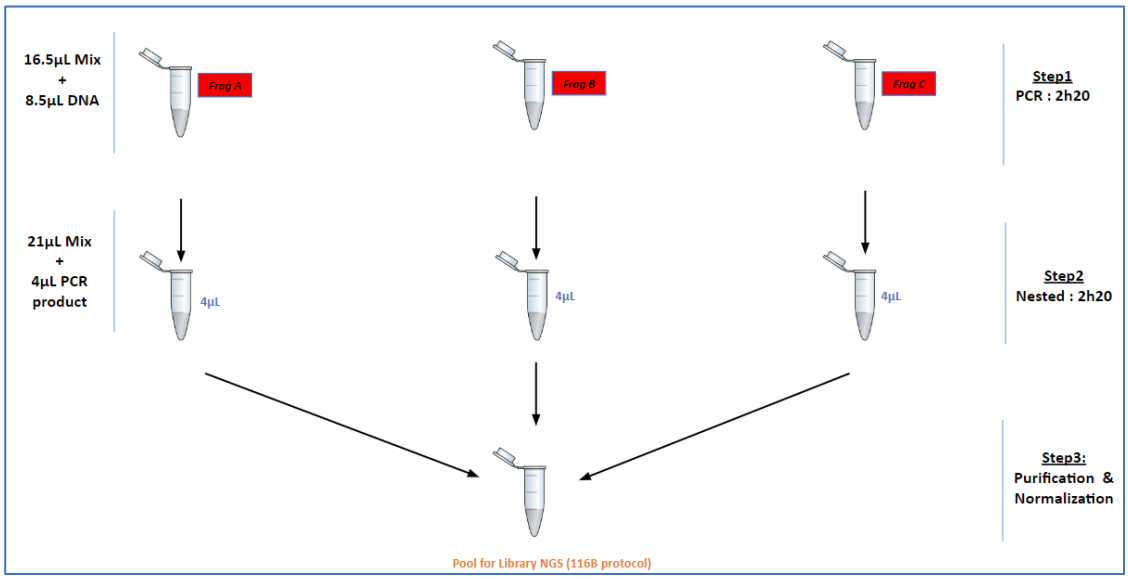

Figure 2: Workflow overview of the DeepChek® Assay Whole Genome HBV Genotyping

Step 1 – PCR reaction

1. Prepare the PCR master mix according to the following table. The PCR master mix typically contains all the components required for PCR reaction except the template DNA. Prepare a volume of PCR master mix greater (n+1) than that required for the total number of reactions to be performed.

Table 2: Reagents for the PCR reaction of the DeepChek® Assay Whole Genome HBV Genotyping

Reagent	Volume for 1 Rn. (PCR step)		
	FRAG 1	FRAG 2	FRAG 3
PCR Master Mix 2X	12.5 µL	12.5 µL	12.5 µL
PCR FRAG 1 (20µM)	4.0 µL		
PCR FRAG 2 (20µM)		4.0 µL	
PCR FRAG 3 (20µM)			4.0 µL
Final Volume	**16.5 µL**	**16.5 µL**	**16.5 µL**

2. Vortex the PCR master mix thoroughly and dispense 16.5 µL into each PCR tube. Mix by pipetting the PCR master mix up and down a few times.
3. Add 8.5 µL of DNA in each PCR tube. Mix by pipetting the master mix up and down a few times.

Step 2 – PCR cycling program

1. Program the thermal cycler according to the program below.

Table 3: RT-PCR cycling program of the DeepChek® Assay Whole Genome HBV Genotyping

Cycle	Temperature (°C)	Time
Activation	95	5 min
PCR - 25 cycles	94	30 sec
	61	1 min
	68	3 min
Final elongation	72	10 min
	10	∞

2. Start the cycling program while PCR tubes are still on ice.
   Δ Safe Stopping Point: After amplification, specimens can be stored overnight at 2–10°C, or at -20°C for long-term storage.
3. **[Recommended]** – Each PCR product can be controlled through electrophoresis on an 2% 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. In that case, first use the DeepChek® Nested PCR reagents.

Note: Expected amplicons size after the RT-PCR steps:

- FRAG 1: ~1460 bp
- FRAG 2: ~829 bp
- FRAG 3: ~1208 bp

Step 3 – Nested PCR reaction (optional)

1. Thaw the PCR product, Nested PCR primers and master mix and place them on ice. Load all the tubes into the centrifuge. Spin the specimens at 11,000 *g* for 10 seconds. And then aspirate and discharge the solution several times before the dispensing.

2. Prepare the Nested PCR master mix according to the table below. The Nested PCR master mix typically contains all the components required for Nested 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 4: Reagents for the Nested RT reaction of the DeepChek® Assay Whole Genome HBV Genotyping

Reagent	Volume for 1 Rn. (Nested PCR step)		
	FRAG 1	FRAG 2	FRAG 3
Nested PCR Master Mix 2X	12.5 µL	12.5 µL	12.5 µL
H2O	4.5 µL	4.5 µL	4.5 µL
Nested PCR FRAG 1 (20µM)	4.0 µL		
Nested PCR FRAG 2 (20µM)		4.0 µL	
Nested PCR FRAG 3 (20µM)			4.0 µL
Final Volume	**21.0 µL**	**21.0 µL**	**21.0 µL**

3. Vortex the Nested PCR master mix thoroughly and dispense 21.0 µL into each PCR tube. Mix by pipetting the Nested PCR master mix up and down a few times.
4. Add 4.0 µL of DNA in each PCR tube. Mix by pipetting the master mix up and down a few times. Program the thermal cycler according to the program below.

Table 5: RT-PCR cycling program of the DeepChek® Assay Whole Genome HBV Genotyping

Cycle	Temperature (°C)	Time
Activation	95	5 min
Nester PCR- 25 cycles	94	30 sec
	61	1 min
	68	3 min
Final elongation	72	10 min
	10	∞

5. Start the cycling program while PCR tubes are still on ice. **Wait until the thermal cycler has reached 95°C. Then place the PCR tubes in the thermal cycler**.

Note: After amplification, specimens can be stored overnight at 2–10°C, or at -20°C for long-term storage.

Note: Expected amplicons size after the Nested PCR steps:

- FRAG 1: ~1460 bp
- FRAG 2: ~829 bp
- FRAG 3: ~1208 bp

RT-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.

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 three (3) fragments are analyzed by a downstream analysis software (e.g., the ABL **DeepChek® Software** (#S-12-023), HBV license and module (REF S-12-023 (BL) and S-12-023 (BM))). 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		Positive control
	Catalog number		Temperature limitation
	Use by		Serial Number
	Manufacturer	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Country and date of manufacturing		Distributor

Contact Information

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