ABL Advanced Biological Laboratories Diagnostics

DEEPCHEK®-HCV ASSAYS & SOFTWARE

NS3 - NS5A - NS5B – Core - 5' UTR

A unique and complete portfolio for HCV genotyping through Sanger & Next Generation (NGS) sequencing

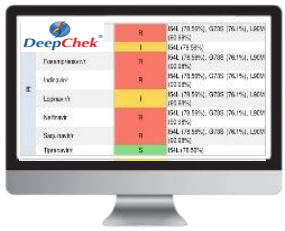
These assays are a unique line of products dedicated to HCV Genotyping and resistance determination through Sanger and NGS sequencing.

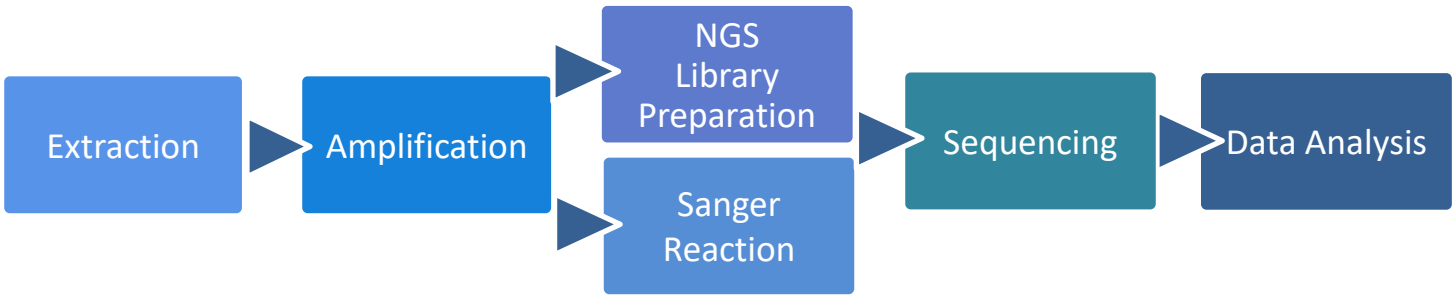
- DeepChek®-HCV NS5B + 5'UTR Genotyping Assay V3
- DeepChek®-HCV NS5B + 5'UTR Genotyping Assay V2.x
- DeepChek®-HCV Core Genotyping Assay V1
- DeepChek®-HCV NS5A DR Assay V1
- DeepChek®-HCV NS3 Genotyping and DR Assay V1
- DeepChek®-HCV NS5B Genotyping and DR Assay V4.x

Workflow overview

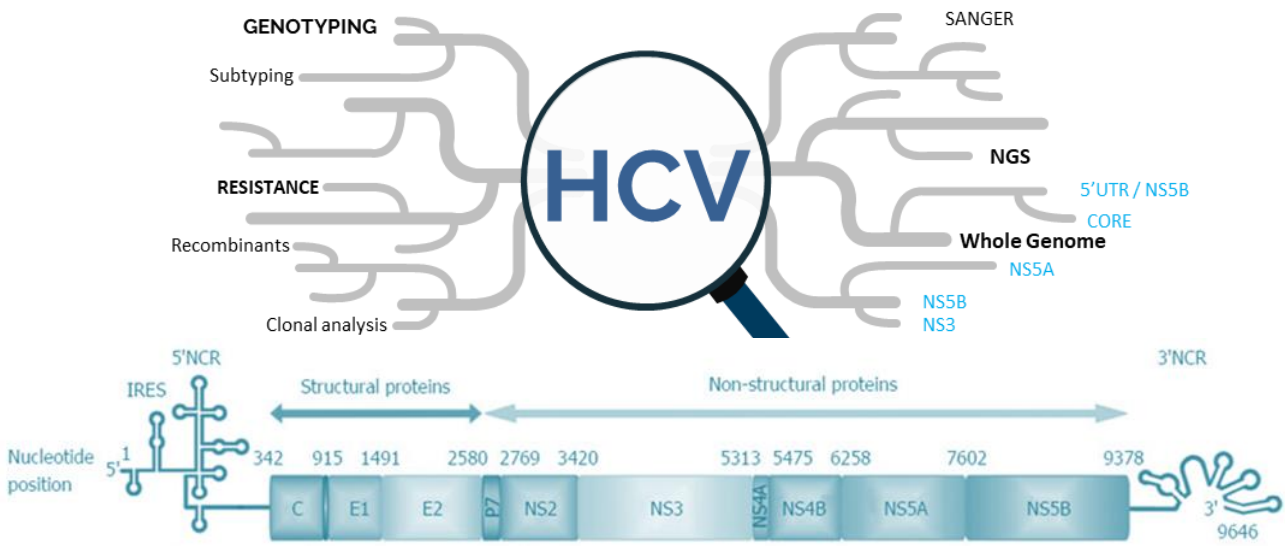








PRODUCT OVERVIEW



DeepChek®-HCV NS5B + 5'UTR Genotyping Assay V3 (Reference 110C24)

- Viral Hepatitis C (HCV) genotyping and subtyping
- Multiplex
- Including Nested PCR for NS5B
- 5'UTR: 244 bp and NS5B: 1048 bp

DeepChek®-HCV NS5A DR Assay V1 (Reference 105A24)

- HCV genotyping and drug susceptibility of a patient's hepatitis C virus (HCV) to the NS5A inhibitors
- Including Nested PCR
- NS5A: codons 1 to 222

DeepChek®-HCV NS5B + 5'UTR Genotyping Assay V2.x (Reference 110B24)

- Viral Hepatitis C (HCV) genotyping and subtyping
- Multiplex
- 5'UTR: 244 bp and NS5B: 420 bp

DeepChek®-HCV NS3 Genotyping and DR Assay V1 (Reference: 108A24)

- HCV genotyping and drug susceptibility of a patient's hepatitis C virus (HCV) to the NS3 inhibitors
- Including Nested PCR
- NS3: codons 1 to 206

DeepChek®-HCV Core Genotyping Assay V1 (Reference 109A24)

- Viral Hepatitis C (HCV) genotyping and subtyping
- Core: 463 bp

DeepChek®-HCV NS5B Genotyping and DR Assay V4.x (Reference: 107D24)

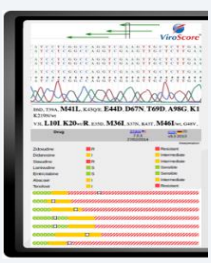
- HCV genotyping and drug susceptibility of a patient's hepatitis C virus (HCV) to the NS5B inhibitors
- Including Nested PCR
- NS5B: codons 1 to 579

HIGHLIGHTS

- TARGET-SPECIFIC AMPLIFICATION (24 samples/kit)
  - Validated on all genotypes (pangenotypic)
  - Sensitivity: 1000 UI/mL from 400 µL plasma/serum
  - Reproducibility >99%
  - PCR instruments also available through reagent rental

- SEQUENCING
  - For Sanger & Next Generation Sequencing (NGS)
  - SANGER: DeepChek® SANGER Sequencing Assay
  - NGS: DeepChek® Library Preparation Assays (incl. 24/48/96/384 indexes)
  - Sanger & NGS sequencing devices also available through reagent rental

- DATA ANALYSIS
  - Sequencing analysis and interpretation with CE-IVD software
  - Sanger (AB1, FASTA) & NGS (FASTA/FASTQ - including paired sequencing, BAM/SAM)
  - Target specific data (5'UTR, NS5B, CORE, NS3, NS5A)
  - Chromatogram editor integrated
  - High resolution subtyping, amino-acid mutations detection, nucleotide changes detection
  - Drug resistance assessment (for NS3, NS5A and NS5B inhibitors) from several up-to-date guidelines
  - Complete and comprehensive reporting
  - Hosting: On premise (local servers) or Cloud (including Health Data Hosting compliance)
  - Servers also available through reagent rental



- EXTRACTION (KITS & INSTRUMENTS)
  - Manual or automated
  - Optimized magnetic beads for fast extraction
  - Easy to prepare and very good performance
  - Fast Protocol : 96 samples within 24 minutes
  - Extraction instrument also available through reagent rental

- AUTOMATION
  - Pipetting robot – open system to be used for pre- or post-PCR automation
  - IT Services (customization, data import, integration / LIS connectivity …)
  - Pipetting Robot also available through reagent rental

HIGHLIGHTS on the DeepChek® technology

ROBUST
CE-IVD and RUO

FLEXIBLE
For low to high throughput (1-384 samples in one run)

FAST
~1-2 days (SANGER)
~2-4 days (NGS)

STANDARDIZED
Kits, Software, Support, Integration with LIMS/HIS, Automation

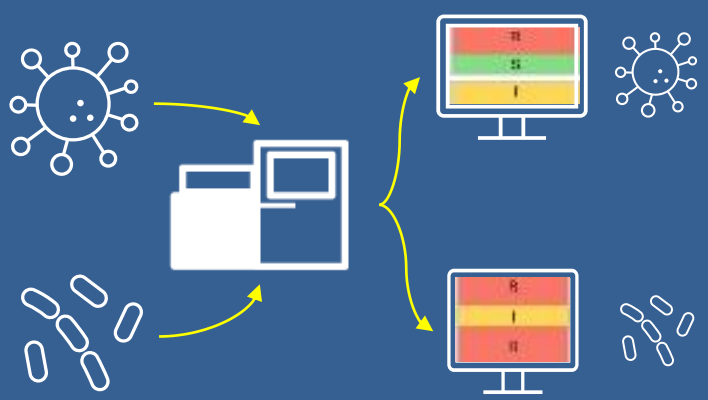
COMPREHENSIVE
Genotyping, Drug Resistance, Tropism, Reporting, Storage…

SECURED
Healthcare Cloud Access, Local installation

POOLING CAPACITY

Pool several DeepChek® Libraries (samples) from different applications in the same NGS run

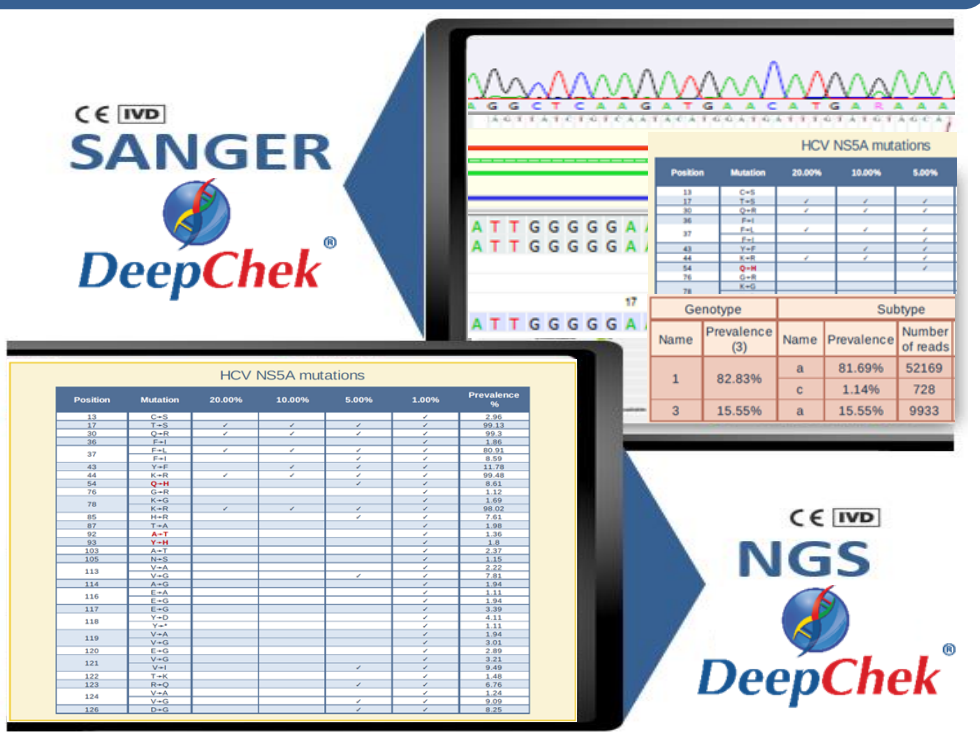
COST EFFECTIVE



- HIV
- SC2
- TB
- HCV
- HBV
- CMV
- HSV
- 16s RNA
- HPV
- BKV
- …

REPORTING

CE IVD SANGER DeepChek®



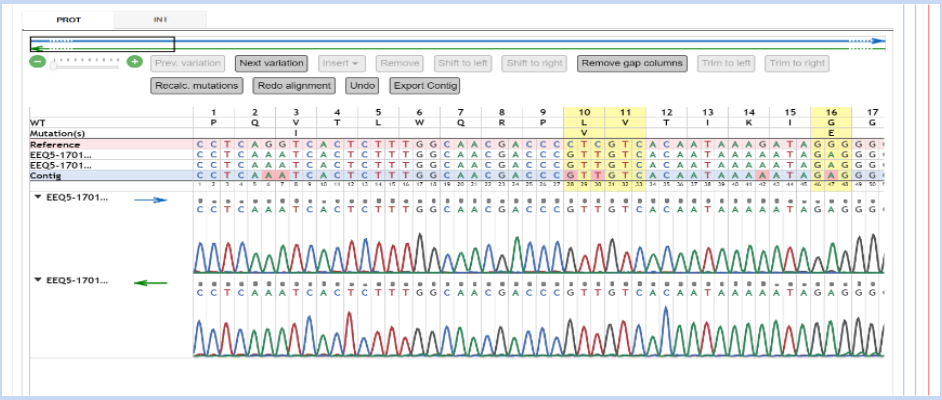
CE IVD NGS DeepChek®

ABL Advanced Biological Laboratories Diagnostics

Improving Disease Management

KEY FEATURES

EMBEDDED CHROMATOGRAM EDITOR



HIGH RESOLUTION SUBTYPING

NS5B (Polymerase)

Genotype		Subtype			
Name	Prevalence (3)	Name	Prevalence	Number of reads	Confidence (1)
1	82.83%	a	81.69%	52169	100%
		c	1.14%	728	100%
3	15.55%	a	15.55%	9933	100%

5'-UTR

Genotype		Subtype			
Name	Prevalence (3)	Name	Prevalence	Number of reads	Confidence (1)
1	82.87%	a	81.71%	52236	100%
		c	1.17%	745	100%
3	15.55%	a	15.55%	9938	100%

MUTATION DETECTION DRUG RESISTANCE ASSESSMENT

HCV NS5A mutations

Position	Mutation	20.00%	10.00%	5.00%	1.00%	Prevalence %
13	C→S				✓	2.96
17	T→S	✓	✓	✓	✓	99.13
30	Q→R	✓	✓	✓	✓	99.3
36	F→I					
37	F→L	✓				
	F→I					
43	Y→F					
44	K→R	✓				
54	Q→H					
76	G→R					
78	K→G					
	K→R	✓				
85	H→R					
87	T→A					
92	A→T					
93	Y→H					
103	A→T					
105	N→S					
113	V→A					
	V→G					
114	A→G					
116	E→A					
	E→G					

HCV Phosphoprotease Inhibitors

	Algorithm	Sanger based sequencing
Daclatasvir	Geno2pheno	R
	International Antiviral Society	I
	Lontok et al. Hepatology 2015	R
Elbasvir (mk-8742)	Geno2pheno	R
	International Antiviral Society	NA
	Lontok et al. Hepatology 2015	NA
Ledipasvir (gs-5885)	Geno2pheno	R
	International Antiviral Society	NA
	Lontok et al. Hepatology 2015	R
Ombitasvir (abt-267)	Geno2pheno	R
	International Antiviral Society	NA
	Lontok et al. Hepatology 2015	R

	Geno2pheno	International Antiviral Society	Lontok et al. Hepatology 2015
S	Susceptible	S	S
I	Possibly resistant Compensatory mutation	I	I
R	Resistant	R	R

OTHER FEATURES

Technology
- Web-based (browser only)
- Software & database
- Local or Cloud (+HDS) hosting
- Unlimited user accounts
- Unlimited analyses

Security
- Data access restriction (pools, read-only mode…)
- Logging of user accesses
- Encrypted database
- Reports validation

Additional features
- Report customization
- Contamination check
- Quality control
- Export (reports, FASTA, XML…)
- Batch mode analysis
- Data mining

Main features
- SANGER data management (AB1, FASTA) with embedded chromatogram editor
- NGS data management (FASTQ) with dedicated pipeline
- Manual or automated base calling
- Variant calling
- Genotyping (per sample & cumulative)
- Subtyping
- Virtual-phenotyping
- Drug resistance through up-to-date guidelines
- Reporting & labelling

Services
- Constant updates
- Annual upgrades (versions)
- Historical data import
- LIMS integration
- HIS integration
- Support
- Trainings

REFERENCES & CONTACT

Product	Reference
DeepChek® Assays	
DeepChek® Assay NS5B / 5'UTR Genotyping V2.x	110B24
DeepChek® Assay NS5B / 5'UTR Genotyping V3	110C24
DeepChek® Assay HCV-CORE Genotyping V1.x	109A24
DeepChek® Assay NS5A Genotyping and Drug Resistance V1.x	105A24
DeepChek® Assay NS5A (GT2) Drug Resistance V1	106A24
DeepChek® Assay NS3 Genotyping and Drug Resistance V1.x	108A24
DeepChek® Assay NS5B Genotyping and Drug Resistance V4.x	107D24
General Laboratory Products	
DeepChek® SANGER SEQUENCING REACTION V2 (24rx/48rx)	123A24/123A48
DeepChek® NGS LIBRARY PREPARATION V1 (24 indexes)	116B24+124B24
DeepChek® NGS LIBRARY PREPARATION V1 (48 indexes)	116B48+124B48
DeepChek® NGS LIBRARY PREPARATION V1 (96 indexes)	116B96+124B96
DeepChek® NGS LIBRARY PREPARATION V1 (384 indexes)	116B384+124B384
DeepChek® NGS Clean-up beads (60mL)	N411-02
DeepChek® 96x0.2 mL well plate (25 units)	B70501-01
Software	
DeepChek® - HCV Software (CE-IVD) Licence	S-12-023 (CL)
DeepChek® - HCV Software (CE-IVD) Module	S-12-023 (CM)

ABL Advanced Biological Laboratories
Diagnostics

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Improving Disease Management

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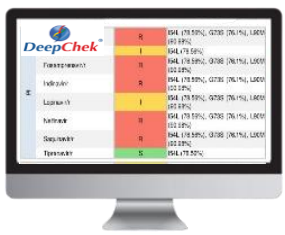
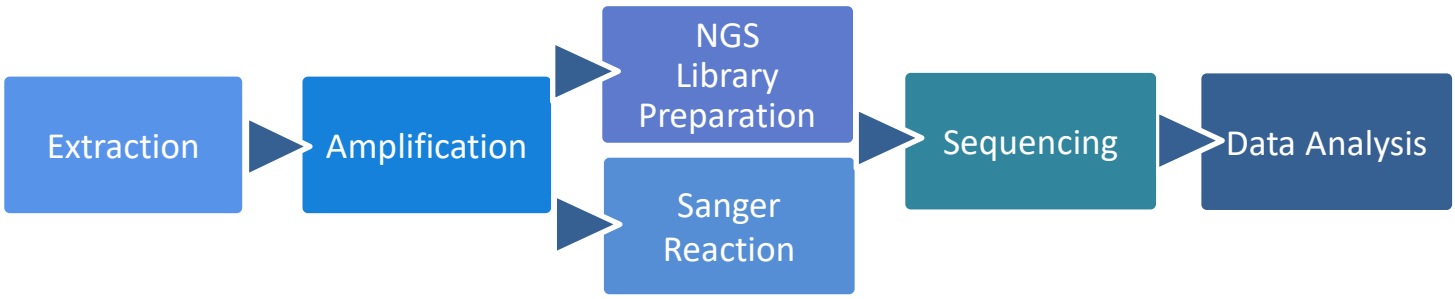
- DeepChek®-HCV NS5B + 5'UTR Genotyping Assay V2.x
- DeepChek®-HCV Core Genotyping Assay V1
- DeepChek®-HCV NS5A DR Assay V1
- DeepChek®-HCV NS3 Genotyping and DR Assay V1
- DeepChek®-HCV NS5B Genotyping and DR Assay V4.x

Workflow overview









PRODUCT OVERVIEW

DeepChek®-HCV NS5B + 5'UTR Genotyping Assay V3 (Reference 110C24)

- HCV genotyping and subtyping
- Specimen: plasma, serum
- LoD: 1000 IU/mL
- Multiplex incl. nested-PCR for NS5B
- amplicon size: 5'UTR: 244 bp and NS5B: 1048 bp
- for NGS and Sanger Sequencing

DeepChek®-HCV NS5A DR Assay V1 (Reference 105A24)

- HCV genotyping and drug susceptibility to the NS5A inhibitors
- Specimen: plasma, serum
- LoD: 1250 IU/mL
- nested-PCR included
- covered region NS5A: codons 1 to 222
- for NGS and Sanger Sequencing

DeepChek®-HCV NS5B + 5'UTR Genotyping Assay V2.x (Reference 110B24)

- HCV genotyping and subtyping
- Specimen: plasma, serum
- LoD: 1250 IU/mL
- Multiplex
- amplicon size 5'UTR: 244 bp and NS5B: 420 bp
- for NGS and Sanger Sequencing

DeepChek®-HCV NS3 Genotyping and DR Assay V1 (Reference: 108A24)

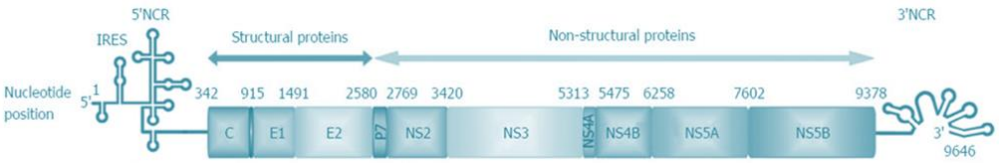
- HCV genotyping and drug susceptibility to the NS3 inhibitors
- Specimen: plasma, serum
- LoD: 1250 IU/mL
- nested-PCR included
- covered region: NS3 codons 1 to 206
- for NGS and Sanger Sequencing

DeepChek®-HCV Core Genotyping Assay V1 (Reference 109A24)

- HCV genotyping and subtyping
- Specimen: plasma, serum
- LoD: 1250 IU/mL
- amplicon size Core: 463 bp
- for NGS and Sanger Sequencing

DeepChek®-HCV NS5B Genotyping and DR Assay V4.x (Reference: 107D24)

- HCV genotyping and drug susceptibility to the NS5B inhibitors
- Specimen: plasma, serum
- LoD: 1250 IU/mL
- nested-PCR included
- covered region NS5B codons 1 to 579
- for NGS and Sanger Sequencing



HIGHLIGHTS

TARGET-SPECIFIC AMPLIFICATION

- Validated on all genotypes (pangenotypic)
- Reproducibility >99%

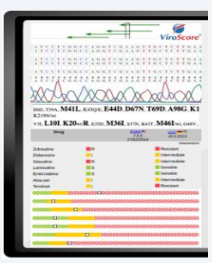
PCR instruments also available through reagent rental

SEQUENCING

- For Sanger & Next Generation Sequencing (NGS)
- SANGER: DeepChek® SANGER Sequencing Assay
- NGS: DeepChek® Library Preparation Assays (24/48/96/384 format)

Sanger & NGS sequencing devices also available through reagent rental

DATA ANALYSIS



- Sequencing analysis and interpretation with CE-IVD software
- Sanger (AB1, FASTA) & NGS (FASTA/FASTQ - including paired sequencing, BAM/SAM)
- Target specific data (5'UTR, NS5B, CORE, NS3, NS5A)
- Chromatogram editor integrated
- High resolution subtyping, amino-acid mutations detection, nucleotide changes detection
- Drug resistance assessment (for NS3, NS5A and NS5B inhibitors) from several up-to-date guidelines
- Complete and comprehensive reporting
- Hosting: On premise (local servers) or Cloud (including Health Data Hosting compliance)

Servers also available through reagent rental

EXTRACTION (KITS & INSTRUMENTS)

- Manual or automated
- Optimized magnetic beads for fast extraction
- Easy to prepare and very good performance
- Fast Protocol : 96 samples within 24 minutes

Extraction instrument also available through reagent rental

AUTOMATION

- Pipetting robot – open system to be used for pre- or post-PCR automation
- IT Services (customization, data import, integration / LIS connectivity …)

Pipetting Robot also available through reagent rental

HIGHLIGHTS on the DeepChek® technology

ROBUST
CE-IVD and RUO

FLEXIBLE
For low to high throughput (1-384 samples in one run)

FAST
~1-2 days (SANGER)
~2-4 days (NGS)

STANDARDIZED
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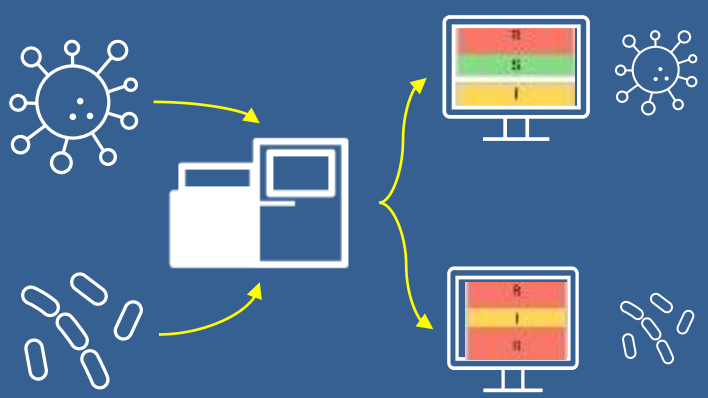
COMPREHENSIVE
Genotyping, Drug Resistance, Tropism, Reporting, Storage…

SECURED
Healthcare Cloud Access, Local installation

POOLING CAPACITY

Pool several DeepChek® Libraries (samples) from different applications in the same NGS run

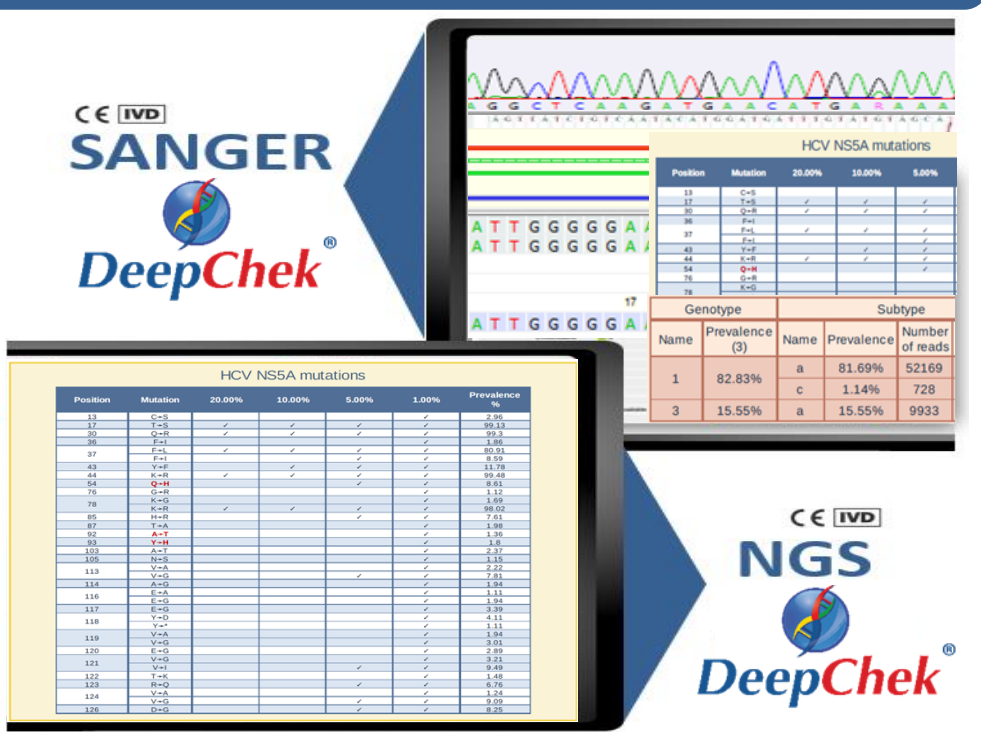
COST EFFECTIVE



- HIV
- SC2
- TB
- HCV
- HBV
- CMV
- HSV
- 16s RNA
- HPV
- BKV
- …

REPORTING

CE IVD SANGER DeepChek®



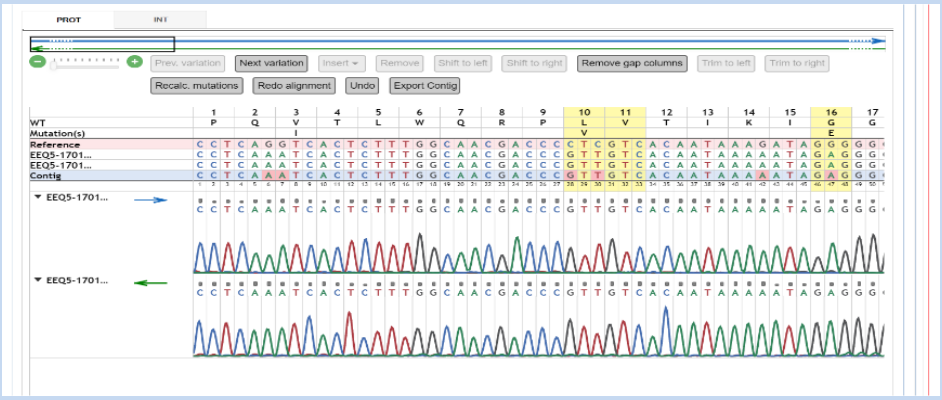
CE IVD NGS DeepChek®

ABL Advanced Biological Laboratories Diagnostics

Improving Disease Management

KEY FEATURES

EMBEDDED CHROMATOGRAM EDITOR



HIGH RESOLUTION SUBTYPING

NS5B (Polymerase)

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5'-UTR

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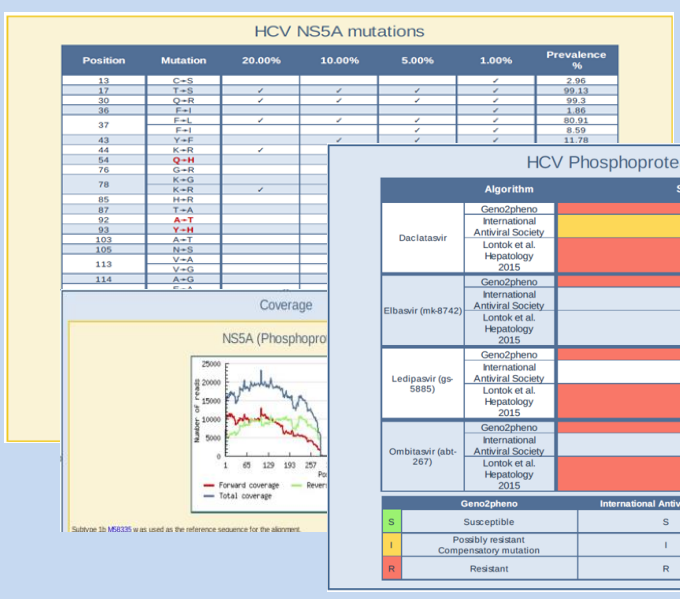
MUTATION DETECTION DRUG RESISTANCE ASSESSMENT



HCV Phosphoprotease Inhibitors

	Algorithm	Sanger based sequencing
Daclatasvir	Geno2pheno	R
	International Antiviral Society	I
	Lontok et al. Hepatology 2015	R
Elbasvir (mk-8742)	Geno2pheno	R
	International Antiviral Society	NA
	Lontok et al. Hepatology 2015	NA
Ledipasvir (gs-5885)	Geno2pheno	R
	International Antiviral Society	NA
	Lontok et al. Hepatology 2015	R
Ombitasvir (abt-267)	Geno2pheno	R
	International Antiviral Society	NA
	Lontok et al. Hepatology 2015	R

	Geno2pheno	International Antiviral Society	Lontok et al. Hepatology 2015
S	Susceptible	S	S
I	Possibly resistant Compensatory mutation	I	I
R	Resistant	R	R

OTHER FEATURES

Technology
- Web-based (browser only)
- Software & database
- Local or Cloud (+HDS) hosting
- Unlimited user accounts
- Unlimited analyses

Security
- Data access restriction (pools, read-only mode…)
- Logging of user accesses
- Encrypted database
- Reports validation

Additional features
- Report customization
- Contamination check
- Quality control
- Export (reports, FASTA, XML…)
- Batch mode analysis
- Data mining

Main features
- SANGER data management (AB1, FASTA) with embedded chromatogram editor
- NGS data management (FASTQ) with dedicated pipeline
- Genotyping (per sample & cumulative)
- Subtyping
- Virtual-phenotyping
- Drug resistance through up-to-date guidelines
- GSS determination and regiment ranking
- Tropism
- Reporting & labelling

Services
- Constant updates
- Annual upgrades (versions)
- Historical data import
- LIMS integration
- HIS integration
- Support
- Trainings

REFERENCES & CONTACT

Product	Reference
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DeepChek® Assay NS5B / 5'UTR Genotyping V3	110C24
DeepChek® Assay HCV-CORE Genotyping V1.x	109A24
DeepChek® Assay NS5A Genotyping and Drug Resistance V1.x	105A24
DeepChek® Assay NS5A (GT2) Drug Resistance V1	106A24
DeepChek® Assay NS3 Genotyping and Drug Resistance V1.x	108A24
DeepChek® Assay NS5B Genotyping and Drug Resistance V4.x	107D24
General Laboratory Products	
DeepChek® SANGER SEQUENCING REACTION V2 (24rx/48rx)	123A24/123A48
DeepChek® NGS LIBRARY PREPARATION V1 (24 indexes)	116B24+124B24
DeepChek® NGS LIBRARY PREPARATION V1 (48 indexes)	116B48+124B48
DeepChek® NGS LIBRARY PREPARATION V1 (96 indexes)	116B96+124B96
DeepChek® NGS LIBRARY PREPARATION V1 (384 indexes)	116B384+124B384
DeepChek® NGS Clean-up beads (60mL)	N411-02
DeepChek® 96x0.2 mL well plate (25 units)	B70501-01
Software	
DeepChek® - HCV Software (CE-IVD) Licence	S-12-023 (CL)
DeepChek® - HCV Software (CE-IVD) Module	S-12-023 (CM)

ABL Advanced Biological Laboratories
Diagnostics

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Improving Disease Management

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Improving Disease Management

Certification of Intended Use

Advanced Biological Laboratories (ABL) S.A declares that the product below is not declared as a medical device neither according to EU Directive 98/79/EC-In Vitro Diagnostics Medical Devices nor to EU Directive 94/42/EEC – Medical Devices.

The product is solely labeled as "RUO", "Research Use Only". It may freely be distributed in the European Economic Area including Luxembourg and other territories.

Manufacturer:
Advanced Biological Laboratories (ABL) S.A,
52-54 Avenue du X Septembre,
2550 Luxembourg, Luxembourg

Catalogue Number	Product Name
113A24	DeepChek® Assay RT Genotyping and Drug Resistance V1

Luxembourg, le 17/11/2021

In kind regards

Ronan Boulmé
ABL - GRC Manager / DPO
Quality Management Representative

As Reviewed by Luxembourg House of Entrepreneurship

The Chamber of Commerce of the Grand Duchy of Luxembourg hereby confirms that the affixed signature in this document corresponds to the specimen of the signature submitted by the company ABL to the Chamber of Commerce

NOV. 18 2021

CHAMBER OF COMMERCE LUXEMBOURG
Tania MARTINS

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2550 Luxembourg, Luxembourg

Catalogue Number	Product Name
114A24	UltraGene Assay VIRAL LOAD V1

Luxembourg, le 17/11/2021

In kind regards

Ronan Boulmé
ABL - GRC Manager / DPO
Quality Management Representative

As Reviewed by Luxembourg House of Entrepreneurship

NOV. 18 2021

The Chamber of Commerce of the Grand Duchy of Luxembourg hereby confirms that the affixed signature in this document corresponds to the specimen of the signature submitted by the company ABL to the Chamber of Commerce

CHAMBER OF COMMERCE LUXEMBOURG
Tania MARTINS

ADVANCED BIOLOGICAL LABORATORIES S.A.
52-54 Avenue du X Septembre
L-2550 Luxembourg
Tel : +352 26 66 93 68
www.ablsa.com

Improving Disease Management

Certification of Intended Use

Advanced Biological Laboratories (ABL) S.A declares that the product below is not declared as a medical device neither according to EU Directive 98/79/EC-In Vitro Diagnostics Medical Devices nor to EU Directive 94/42/EEC – Medical Devices.

The product is solely labeled as "RUO", "Research Use Only". It may freely be distributed in the European Economic Area including Luxembourg and other territories.

Manufacturer:
Advanced Biological Laboratories (ABL) S.A,
52-54 Avenue du X Septembre,
2550 Luxembourg, Luxembourg

Catalogue Number	Product Name
168A	DeepChek® HCV Genotyping External Controls V1

Luxembourg, le 17/11/2021

In kind regards

Ronan Boulmé
ABL - GRC Manager / DPO
Quality Management Representative

As Reviewed by Luxembourg House of Entrepreneurship

NOV. 18 2021

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Manufacturer:
Advanced Biological Laboratories (ABL) S.A,
52-54 Avenue du X Septembre,
2550 Luxembourg, Luxembourg

Catalogue Number	Product Name
184A24	DeepChek® Assay Whole Genome HBV Genotyping V1

Luxembourg, le 09/06/2022

In kind regards

Ronan Boulmé
ABL - GRC Manager / DPO
Quality Management Representative

As Reviewed by Luxembourg House of Entrepreneurship

15 JUIN 2022
The Chamber of Commerce of the Grand Duchy of Luxembourg hereby confirms that the affixed signature in this document corresponds to the specimen of the signature submitted by the company ABL to the Chamber of Commerce
CHAMBER OF COMMERCE LUXEMBOURG
Melissa RAMDEDOVIC

ADVANCED BIOLOGICAL LABORATORIES S.A.
52-54 Avenue du X Septembre
L-2550 Luxembourg
Tel : +352 26 66 93 68
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Improving Disease Management

Certification of Intended Use

Advanced Biological Laboratories (ABL) S.A declares that the product below is not declared as a medical device neither according to EU Regulation 2017/746 - In Vitro Diagnostics Medical Devices nor to EU Regulation 2017/745 – Medical Devices.

The product is solely labeled as "RUO", "Research Use Only". It may freely be distributed in the European Economic Area including Luxembourg and other territories.

Manufacturer:
Advanced Biological Laboratories (ABL) S.A,
52-54 Avenue du X Septembre,
2550 Luxembourg, Luxembourg

Catalogue Number	Product Name
199A24	DeepChek® Assay Whole Genome HDV Genotyping V1.x

Luxembourg, le 14/09/2023

In kind regards,

Dr Chalom B. Sayada
Administrateur Délégué
ABL S.A.

As Reviewed by Luxembourg House of Entrepreneurship

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26AF-7E13-20C6

Visé par la CHAMBRE de COMMERCE du GRAND-DUCHÉ de LUXEMBOURG
VISA
Nr. V 83 2023 00624

Grand Duché du Luxembourg
14/09/2023

Camille Schneider

ADVANCED BIOLOGICAL LABORATORIES S.A.
52-54 Avenue du X Septembre
L-2550 Luxembourg
Tel : +352 26 66 93 68
www.ablsa.com

Improving Disease Management

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The product is solely labeled as "RUO", "Research Use Only". It may freely be distributed in the European Economic Area including Luxembourg and other territories.

Manufacturer:
Advanced Biological Laboratories (ABL) S.A,
52-54 Avenue du X Septembre,
2550 Luxembourg, Luxembourg

Catalogue Number	Product Name
113B24	DeepChek® Assay RT Genotyping and Drug Resistance V2.x

Luxembourg, on 18th of September 2025

In kind regards,

Dr Chalom B. Sayada
Administrateur Délégué
ABL S.A.

As Reviewed by Luxembourg House of Entrepreneurship

Grand Duché du Luxembourg
18/09/2025

Camille Schneider

DeepChek®-HBV/HDV Clinical Genotyping report

DeepChek®-HBV/HDV analysis summary

Patient/Sample information

Patient data	
Viral Load	
Viral Load Date	
Viral Load Method	
Past treatments	
Last treatment	
ALT	0 UI/L
HBe Ag	Missing data
Report id	102-a24a1d1c-992c-11e8-8e7a-46e0b167dabb

Sample ID	13FTO6_S17_NGS
Alternative ID	
Sample type	Plasma
Sample date	21/11/2017
Reason for genotyping analysis	Drug resistance

Input

RT, PreS1, PreS2, HBsAg, BCP, PC, TP, Spacer, RNaseH, X, URR, Core :

HBV4_S17_L001_R1_001.fastq

HBV4_S17_L001_R2_001.fastq

=> 99.91% of the 126678 initial reads mapped to HBV organism

Comments

NGS details

Date of sequencing	27/07/2018
NGS Method	Homebrew
Assay version	
Plate ID	
Cartridge S/N	
Reagent expiration date	
Notes	

DeepChek®-Sanger Information

Not available

DeepChek®-HBV/HDV analysis details

Sequencing platform	Illumina - MiSeq
Processing software version	Missing data
Processing started date	06/08/2018 05:51:56
Processing finished date	
Coverage	
Polymerase	1-344
PreS1	1-119
PreS2	1-55
Surface Antigene	1-226
Basal Core Promoter	1-107 (nt)
Precore	1-29
Terminal protein	1-179
Spacer	1-169
RNaseH	1-154
X	1-155
Upper regulatory region	1-130 (nt)
Core	1-186

DeepChek®-HBV/HDV software version	2.0
DeepChek®-HBV/HDV expert system	2.2
DeepChek®-HBV/HDV algorithms version	9.0

EASL (2017 2017)

Geno2Pheno (2.0 2009)

Grade (2007 08/2007)

HBVDB (43.0 05-2018)

SeqHepB (6 November 2016)

Classification of mutations of interest: Geno2pheno 2009

Report id	102-a24a1d1c-992c-11e8-8e7a-46e0b167dabb	*Sample ID*	13FTO6_S17_NGS
		Sample date	21/11/2017

DeepChek®-HBV/HDV Drug Resistance Determination

HBV reverse transcriptase domain Inhibitors

	Algorithm	20.00%	5.00%
Adefovir	EASL	R	R
	Geno2Pheno	R	R
	Grade	R	R
	HBVDB	R	R
	SeqHepB	R	R
Entecavir	EASL	S	S
	Geno2Pheno	S	S
	Grade	S	S
	HBVDB	S	S
	SeqHepB	S	S
Lamivudine	EASL	I	I
	Geno2Pheno	R	R
	Grade	S	S
	HBVDB	R	R
	SeqHepB	R	R
Telbivudine	EASL	I	I
	Geno2Pheno	R	R
	Grade	S	S
	HBVDB	R	R
	SeqHepB	R	R
Tenofovir	EASL	I	I
	Geno2Pheno	I	I
	Grade	S	S
	HBVDB	I	I
	SeqHepB	R	R

	EASL	Geno2Pheno	Grade	HBVDB	SeqHepB
S	Sensitive	Susceptible	Susceptible Hypersusceptibility	Sensitive	Sensitive
I	Intermediate Reduced response	Limited susceptibility Compensatory mutation Partly resistant	Limited susceptibility Intermediate	Intermediate	Reduced sensitivity
R	Resistant	Resistant	Resistance	Resistant	Resistant

Risk of developing AdLD (Advanced Liver Disease)

Risk Level	Risk Level Associated Mutations
Increased risk	bcpA20T, bcpG22A

Vaccine/immune escape

Vaccine/immune escape
No clinically significant changes detected

DeepChek®-HBV/HDV Mutation Analysis

HBV reverse transcriptase domain mutations

Position	Mutation	20.00%	5.00%	Prevalence %
7	D→G	✓	✓	99.83
8	E→A	✓	✓	62.82
30	V→A		✓	9.74
38	A→T	✓	✓	34.58
53	I→V	✓	✓	63.03
	I→S	✓	✓	30.28
54	S→T	✓	✓	97.86
128	T→A	✓	✓	65.46
181	**A→V**	✓	✓	32.83
	A→T	✓	✓	65.83
219	S→A	✓	✓	99.55
236	**N→T**	✓	✓	99.66
275	K→N	✓	✓	66.16
319	Q→K	✓	✓	99.62

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Pre-S1 mutations

Position	Mutation	20.00%	5.00%	Prevalence %
5	S→T	✓	✓	99.59
48	V→N	✓	✓	65.02
	V→I	✓	✓	34.71
51	D→T	✓	✓	63.6
	D→H	✓	✓	35.04
56	N→K	✓	✓	64.51
66	R→G	✓	✓	99.75
74	I→L	✓	✓	99.68
85	L→S	✓	✓	63.44

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Pre-S2 mutations

Position	Mutation	20.00%	5.00%	Prevalence %
11	T→A	✓	✓	99.9
54	T→A	✓	✓	99.77
55	N→H	✓	✓	62.52

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Surface mutations

Position	Mutation	20.00%	5.00%	Prevalence %
14	V→A	✓	✓	35.21
	V→G	✓	✓	64.56
21	L→S	✓	✓	34.56
40	N→S	✓	✓	31.3
44	G→E	✓	✓	28.1
45	S→A	✓	✓	34.91
49	L→R	✓	✓	98.35
100	Y→C	✓	✓	64.17
131	N→wt	✓	✓	99.55
161	Y→F	✓	✓	99.82
172	W→*	✓	✓	65.84
173	L→F	✓	✓	32.77
194	A→V	✓	✓	33.22
210	S→R	✓	✓	99.72
217	P→L	✓	✓	67.03

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Basal Core Promoter mutations

Position	Mutation	20.00%	5.00%	Prevalence %
20	a→t	✓	✓	99.72
22	g→a	✓	✓	99.49
23	t→c	✓	✓	99.89
48	c→t	✓	✓	99.83
84	c→g			4.27

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Precore mutations

Position	Mutation	20.00%	5.00%	Prevalence %
13	T→S	✓	✓	99.75
29	G→D	✓	✓	99.44

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Reverse Transcriptase TP mutations

Position	Mutation	20.00%	5.00%	Prevalence %
36	H→N	✓	✓	99.49
71	I→V	✓	✓	31.17
95	Q→E	✓	✓	69.43
165	T→S	✓	✓	65.64

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Spacer mutations

Position	Mutation	20.00%	5.00%	Prevalence %
8	V→D	✓	✓	99.55
10	K→Q	✓	✓	99.68
24	S→P	✓	✓	33.61
51	R→N	✓	✓	34.61
	R→Q	✓	✓	65.1
54	R→P	✓	✓	35.58
	R→H	✓	✓	63.43
60	P→T	✓	✓	64.41
61	S→G	✓	✓	99.9
69	K→R	✓	✓	99.48
77	Y→S	✓	✓	99.12
106	K→T	✓	✓	32.68
109	Y→H	✓	✓	33.74
130	L→I	✓	✓	63.42
133	N→S	✓	✓	99.76

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Ribonuclease H mutations

Position	Mutation	20.00%	5.00%	Prevalence %
25	M→L	✓	✓	30.43
77	T→A	✓	✓	99.74
108	S→Y	✓	✓	99.69
138	V→D	✓	✓	99.53
143	A→D	✓	✓	68.85

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV X mutations

Position	Mutation	20.00%	5.00%	Prevalence %
6	Y→C	✓	✓	99.5
22	G→S	✓	✓	99.76
87	Q→R	✓	✓	32.03
88	I→S	✓	✓	99.88
130	K→M	✓	✓	99.72
131	V→T	✓	✓	99.37
151	F→L			4.57

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Upper regulatory mutations

Position	Mutation	20.00%	5.00%	Prevalence %
21	a→g	✓	✓	32.05
24	t→g	✓	✓	99.98
115	g→a	✓	✓	99.96

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Mutation Analysis

HBV Core mutations

Position	Mutation	20.00%	5.00%	Prevalence %
51	H→R			3.18
63	G→V	✓	✓	98.62
77	E→D	✓	✓	99.53
93	V→M	✓	✓	99.55
130	P→Q	✓	✓	99.65
171	P→Q	✓	✓	99.61
183	S→P	✓	✓	98.9

Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Mutations of interest based on Geno2pheno (Bold red text)

Insufficient number of sequences to guarantee, at the 99% confidence level, that all mutations with the given threshold frequency have been found at that position.

DeepChek®-HBV/HDV Subtyping

Polymerase

Genotype	Prevalence	Number of reads	Confidence
A	96.53%	44154	98.14%
A or D	1.46%	667	96.27%
Total count : 45743			

PreS1

Genotype	Prevalence	Number of reads	Confidence
A	95.59%	18394	96.73%
A or C	4.25%	818	94.95%
Total count : 19243			

PreS2

Genotype	Prevalence	Number of reads	Confidence
A	99.97%	10537	98.11%
Total count : 10540			

Surface Antigene

Genotype	Prevalence	Number of reads	Confidence
A	94.96%	29846	97.98%
A or D	2.12%	667	96.27%
Total count : 31429			

Basal Core Promoter

Genotype	Prevalence	Number of reads	Confidence
A	99.69%	1616	97.55%
Total count : 1621			

Precore

Genotype	Prevalence	Number of reads	Confidence
A	74.8%	1508	98.75%
A or D or E	19.59%	395	98.51%
D or E or H	2.53%	51	98.04%
A or D	2.53%	51	98.48%
Total count : 2016			

Terminal protein

Genotype	Prevalence	Number of reads	Confidence
A	98.08%	25633	97.64%
A or D	1.19%	312	96.46%
Total count : 26136			

Spacer

Genotype	Prevalence	Number of reads	Confidence
A	96.66%	24580	97.09%
A or C	3.22%	818	94.95%
Total count : 25430			

RNaseH

Genotype	Prevalence	Number of reads	Confidence
A	99.52%	19578	98.69%
Total count : 19672			

X

Genotype	Prevalence	Number of reads	Confidence
A	99.33%	13557	98.26%
Total count : 13649			

Upper regulatory region

Genotype	Prevalence	Number of reads	Confidence
A	99.96%	5103	98.29%
Total count : 5105			

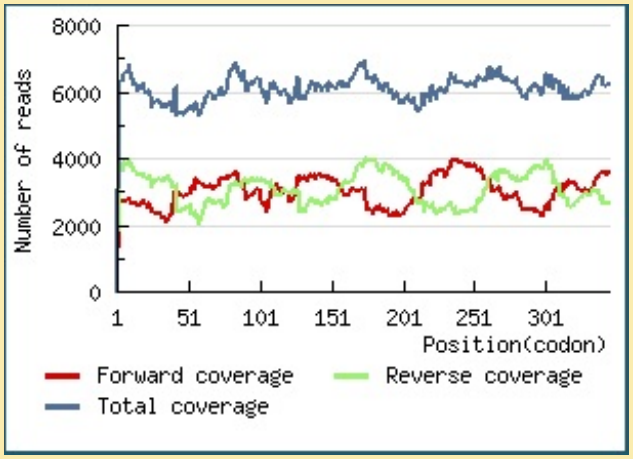
Core

Genotype	Prevalence	Number of reads	Confidence
A	95.66%	22382	97.42%
A or D or E	1.69%	395	98.51%
A or D	1.55%	363	96.75%
Total count : 23398			

DeepChek®-HBV/HDV Expert System

Coverage

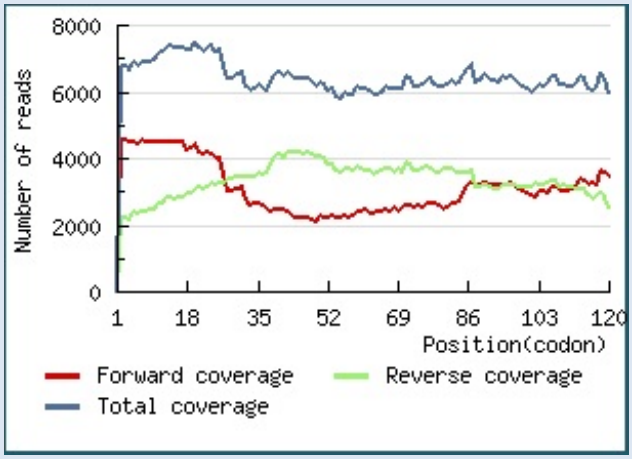
Polymerase



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

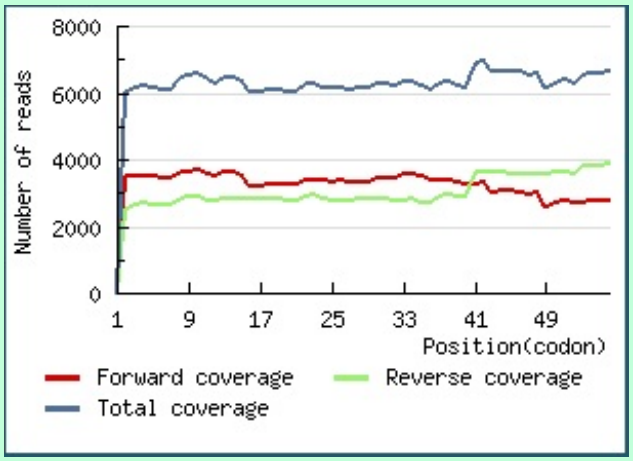
PreS1



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

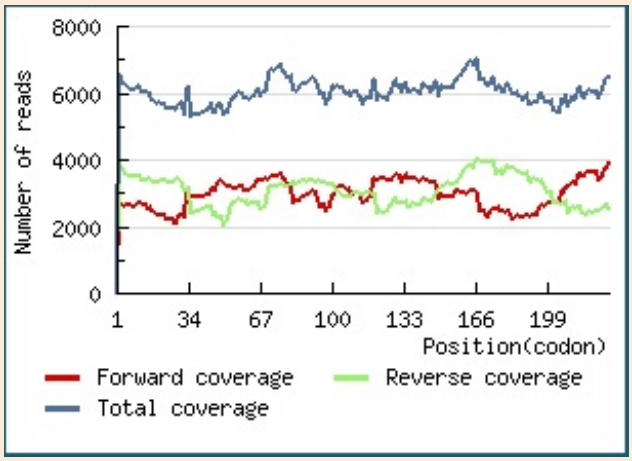
PreS2



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

Surface Antigene



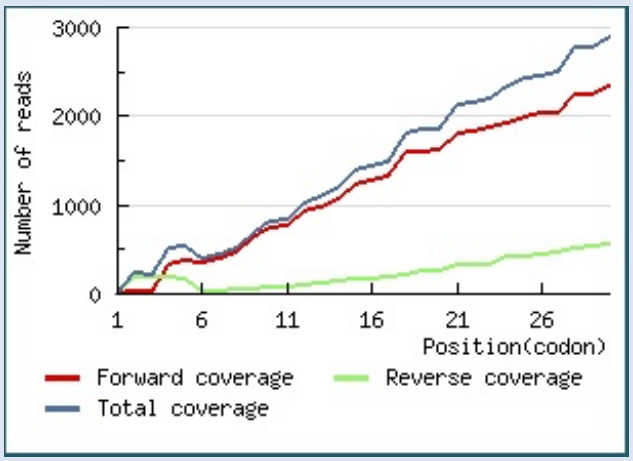
Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

DeepChek®-HBV/HDV Expert System

Coverage

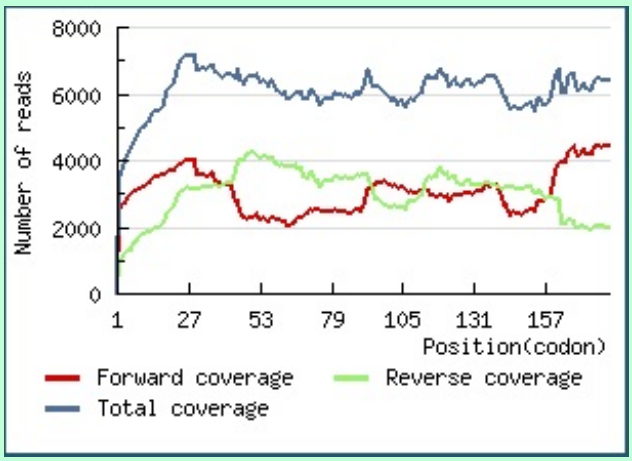
Precore



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

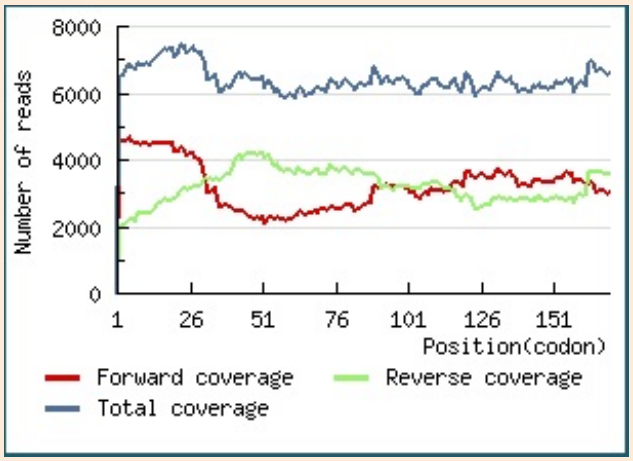
Terminal protein



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

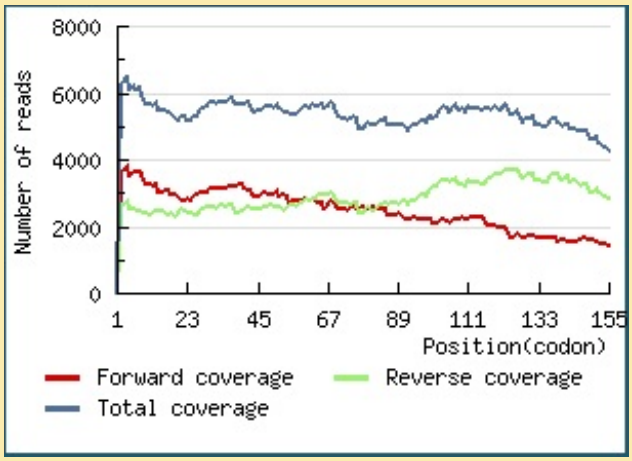
Spacer



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

RNaseH



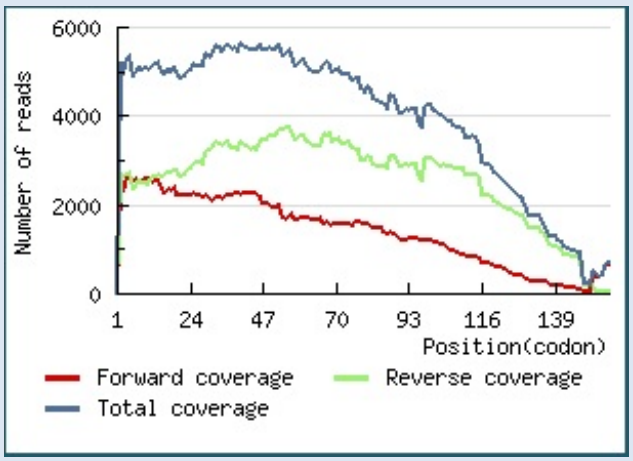
Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

DeepChek®-HBV/HDV Expert System

Coverage

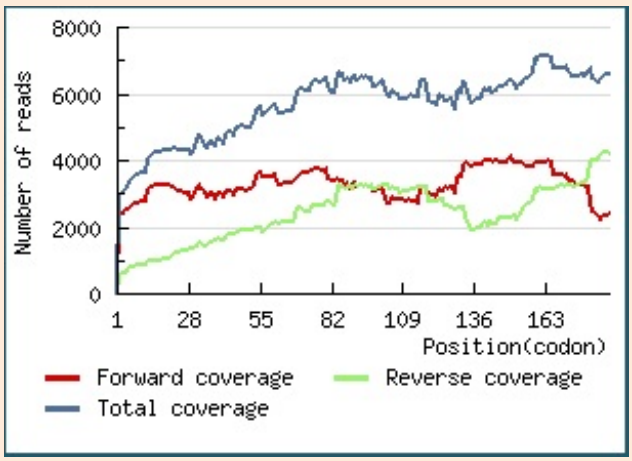
X



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

Core



Subtype A X02763 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
20.00%	23	
5.00%	92	

DeepChek®-HBV/HDV Expert System

Discarded mutations

Polymerase

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	1968 mutations (see details on Quality information report)
Coverage filtering	1449 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	181 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	

PreS1

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	625 mutations (see details on Quality information report)
Coverage filtering	486 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	13 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	

PreS2

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	276 mutations (see details on Quality information report)
Coverage filtering	222 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	8 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	

Surface Antigene

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	1304 mutations (see details on Quality information report)
Coverage filtering	916 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	151 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	

Basal Core Promoter

Reasons excluded	Mutations
Coverage filtering	73 mutations (see details on Quality information report)
Noisy mutations filtering (Threshold : 3%)	4 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	15 mutations (see details on Quality information report)

Precore

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	60 mutations (see details on Quality information report)
Coverage filtering	56 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	
Forward/Reverse unbalanced coverage	11 mutations (see details on Quality information report)

Terminal protein

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	921 mutations (see details on Quality information report)
Coverage filtering	739 mutations (see details on Quality information report)
Forward/Reverse	13 mutations (see details on Quality

Spacer

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	886 mutations (see details on Quality information report)
Coverage filtering	669 mutations (see details on Quality information report)
Forward/Reverse	28 mutations (see details on Quality

unbalanced frequency	information report)
Forward/Reverse unbalanced coverage	

unbalanced frequency	information report)
Forward/Reverse unbalanced coverage	

RNaseH

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	730 mutations (see details on Quality information report)
Coverage filtering	559 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	11 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	

X

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	591 mutations (see details on Quality information report)
Coverage filtering	464 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	1 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	4 mutations (see details on Quality information report)

Upper regulatory region

Reasons excluded	Mutations
Coverage filtering	198 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	1 mutations (see details on Quality information report)
Noisy mutations filtering (Threshold : 3%)	48 mutations (see details on Quality information report)

Core

Reasons excluded	Mutations
Noisy mutations filtering (Threshold : 3%)	910 mutations (see details on Quality information report)
Coverage filtering	727 mutations (see details on Quality information report)
Forward/Reverse unbalanced frequency	19 mutations (see details on Quality information report)
Forward/Reverse unbalanced coverage	

DeepChek®-HBV/HDV References

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3. "Emergence of a novel lamivudine-resistant hepatitis B virus variant with a substitution outside the YMDD motif.", Yatsuji H., Noguchi C., Hiraga N., Mori N., Tsuge M., Imamura M., Takahashi S., Iwao E., Fujimoto Y., Ochi H., Abe H., Maekawa T., Tateno C., Yoshizato K., Suzuki F., Kumada H., Chayama K. Antimicrob Agents Chemother., 50: 3867-3874, 2006.
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7. "Use of the DeepChek®-HIV system in the PRIUS study: validation of a new reliable genotyping solution to streamline the 454 sequencing analysis of HIV drug resistance in routine diagnostics and research applications", R. Paredes et al. International Workshop on HIV & HEPATITIS VIRUS – Drug Resistance and Curative Strategies - JUNE 5-9, 2012 | Sitges, Spain.
8. "DeepChek® HIV v1.0., a reliable tool for the bioinformatics analysis and resistance interpretation of Massive Ultra Deep Sequencing of HIV genomes", F. Garcia et al. 10th European meeting on HIV & Hepatitis (Barcelona, March 2012).
9. Li H. and Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics, 25:1754-60. [PMID: 19451168]

Signature

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DeepChek® Assay

RT Genotyping and Drug Resistance (RUO)

V1

User Guide

Σ
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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)**

Publication number: IFU_113A24_EN_RUO_V1_R1

March 2022

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

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.

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

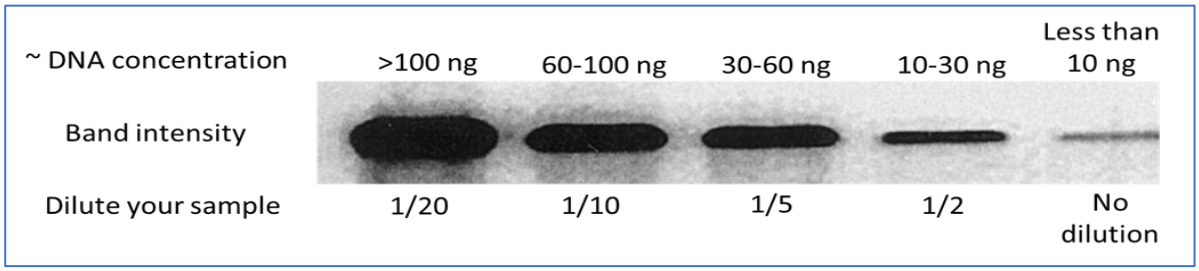
6. Start the DeepChek® Assay RT cycling program while PCR tubes are still on ice. After amplification, samples can be stored overnight at 2–10°C, or at –20°C for long-term storage.
7. **[Recommended]** - PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of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

17 rue des Jardiniers, L-1835 Luxembourg, Luxembourg

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the device. 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

Effective date: 28th March 2022

DeepChek® Assay

RT Genotyping and Drug Resistance (RUO)

V1

User Guide

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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.

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

Publication number: IFU_113A24_EN_RUO_V1_R0

May 2021

Contents

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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 analysed 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
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H_2O	1 x 500 µL	Blue	-25°C to - 15 °C
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*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 in order 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.
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- 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 1250 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:

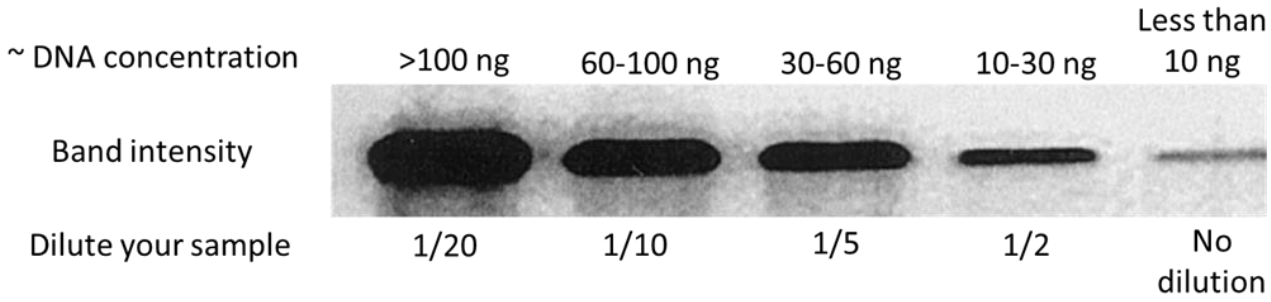
- **G Fragment: 1563 bp**
- **H Fragment: 760 bp**

SingleRound 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 to perform 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	10 sec
	50	5 sec
	60	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	10 sec
	50	5 sec
	60	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

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

Through Illumina MiSeq

- **116A24 / 116A48 / 116A96 | DeepChek® NGS LIBRARY PREPARATION V1 (24 / 48 or 96 reactions).**
- MS-103-1003 | MiSeq Reagent Nano kit, v2 (500 cycles).

Through 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

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

17 rue des Jardiniers, L-1835 Luxembourg, Luxembourg

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 1.0

Effective date: 10th May 2021

DeepChek® Assay

RT Genotyping and Drug Resistance (RUO)

Σ
24

User Guide

Version 1 – Revision 2

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) GTIN: 05407007960941**

Publication number: IFU_113A24_EN_RUO_V1_R2

May 2023

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
05/05/2023	A	1.2	• Modification of assay components

Contents

Application

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.

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 (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	1 x 800 µL	Green	-25°C to - 15 °C
G Frag HBV RT-FOR Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
G Frag HBV RT-REV Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
Sequencing G Frag HBV RT-FOR Primers	1 x 35 µL	Red	-25°C to - 15 °C
Sequencing G Frag HBV RT-REV Primers	1 x 35 µL	Red	-25°C to - 15 °C
H Frag HBV RT-FOR Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
H Frag HBV RT-REV Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
Sequencing H Frag HBV RT-FOR Primers	1 x 35 µL	Red	-25°C to - 15 °C
Sequencing H Frag HBV RT-REV Primers	1 x 35 µL	Red	-25°C to - 15 °C
H_2O	1 x 500 µL	Blue	-25°C to - 15 °C

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

Reagent storage and handling

The ***DeepChek® Assay RT (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 (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	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

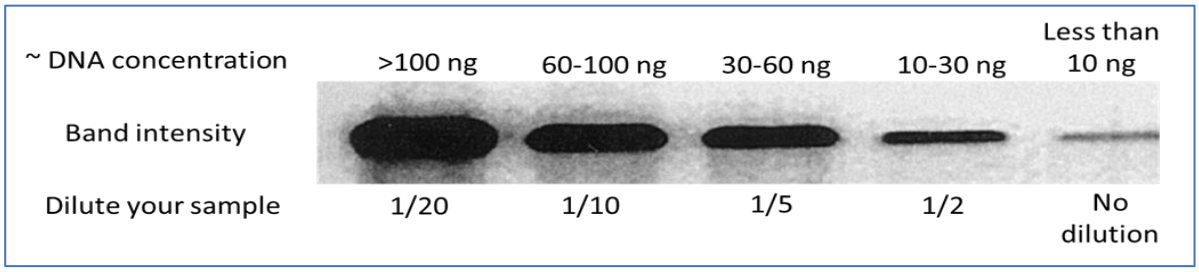
6. Start the DeepChek® Assay RT cycling program while PCR tubes are still on ice. After amplification, samples can be stored overnight at 2–10°C, or at –20°C for long-term storage.
7. **[Recommended]** - PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of 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 (**116BX**).

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.
52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the device. 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 1.2

Effective date: 05th May 2023

DeepChek® Assay

RT Genotyping and Drug Resistance (RUO)

24

User Guide

Version 1 – Revision 3

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)**

GTIN: 05407007960941

Publication number: IFU_113A24_EN_RUO_V1_R3

August 2023

Document control

Date	Device version	IFU version	Description of change
18/08/2023	A	1.3	• Review and text update for Application, Principle of the Assay sections • Add Note after assay components • Typo change: DNA instead of RNA • Simplification of the Sanger sequencing section
05/05/2023	A	1.2	• Modification of assay components
28/03/2022	A	1.1	• Change of the analytical sensitivity to 1000 UI/mL in DNA extraction section • Update the list of next generation sequencing instruments and reagents

Contents

Application

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.

The DeepChek® Assay RT genotyping and Drug Resistance (DR) (RUO) kit utilizes PCR technology for amplifying relevant portions of the Hepatitis B Virus (HBV) Reverse Transcriptase (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, capillary electrophoresis (Sanger) and next generation sequencing (NGS) workflow.

Principles of the assay

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

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

Label	Volume for 24 Rxn	Color cap	Storage
Master Mix	1 x 800 µL	Green	-25°C to - 15 °C
G Frag HBV RT-FOR Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
G Frag HBV RT-REV Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
Sequencing G Frag HBV RT-FOR Primers	1 x 35 µL	Red	-25°C to - 15 °C
Sequencing G Frag HBV RT-REV Primers	1 x 35 µL	Red	-25°C to - 15 °C
H Frag HBV RT-FOR Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
H Frag HBV RT-REV Primers	1 x 45 µL	Yellow	-25°C to - 15 °C
Sequencing H Frag HBV RT-FOR Primers	1 x 35 µL	Red	-25°C to - 15 °C
Sequencing H Frag HBV RT-REV Primers	1 x 35 µL	Red	-25°C to - 15 °C
H_2O	1 x 500 µL	Blue	-25°C to - 15 °C

Note: G and H are not HBV gene names. PCR Primer sets will amplify the RT gene.

Reagent storage and handling

The ***DeepChek® Assay RT (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.

DNA 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 (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.

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

PCR Reagent	G Frag Volume	H Frag Volume
Master Mix	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

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**.

Table 3: G and H fragments PCR Cycling Program

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	∞

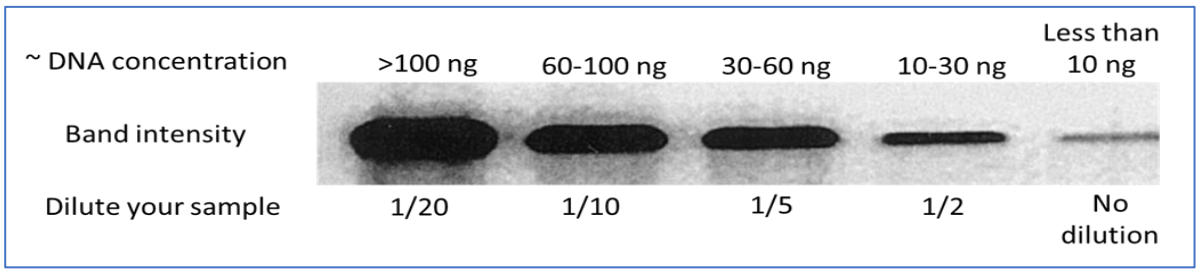
6. Start the DeepChek® Assay RT cycling program while PCR tubes are still on ice. After amplification, samples can be stored overnight at 2–10°C, or at –20°C for long-term storage.
7. **[Recommended]** - PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of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

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 DNA 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 PCR product before sequencing.

For G and H fragments use the DeepChek® SANGER SEQUENCING REACTION (RUO) available in 24 reactions (REF 123A24 / GTIN: 05407007960057) and 48 reactions (REF 123A48 / GTIN: 05407007960040). H

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 (**116BX**).

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: 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

Advanced Biological Laboratories (ABL) S.A.
52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the device. 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 1.3

Effective date: 18th August 2023

DeepChek®

HCV Genotyping External Controls

V1.X *(RUO)*

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.

REF **168A**

Publication number:IFU_168A_RUO_EN_V1_R0 IFU_168A_RUO_EN_V1_R0

November 2021

Contents

Application

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 is ready to use, composed of full process controls, designed to evaluate performance of molecular tests. The product can be used for verification of the ABL DeepChek® assays, training of laboratory personnel for the set-up of the ABL DeepChek® assays and to monitor ABL DeepChek® assays-kit lot performance.

These controls are sold as consumable testing materials only with ABL DeepChek® assays.

Principles of the assay

The controls are inactivated and purified extracted RNA, non-infectious, from isolates (HCV Strains from genotypes 1, 3 and 4), with various concentrations (IU/mL) of HCV. The product is needed to ensure the PCR reactions setup and reagents integrity. The control is ready-to-use in the PCR reactions and shall not be included during extraction.

Assay components

Label	Volume	Color cap
HCV1	20 µL	Clear
HCV2	20 µL	Clear
HCV3	20 µL	Clear
HCV4	20 µL	Clear
HCV5	20 µL	Clear

*Table 1: Volumes and storage conditions of the **DeepChek® HCV Genotyping External Controls V1.X (RUO)***

Reagent storage and handling

The controls should be stored at -65 °C or below.

Materials required but not provided

A “no template” (negative) control (NTC) consisting of Water (molecular grade) shall be used and is needed to detect cross-contamination during all reaction steps and to determine validity of the test run.

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.
- Repetitive freezing and thawing is not recommended. Titer will be altered by multiple freeze-thaws.
- Quality control materials should be used in accordance with local, state, federal, and accreditation requirements.
- HCV is a Biosafety Level 2 organism. It must be used within a Biological Safety Level 2 facility or cabinet. Please consult your institution’s regulations regarding the use of this product. For a detailed discussion on biological safety see the 5th edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL), published by the CDC at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.
- Each laboratory must evaluate the product and establish their own acceptance criteria.
- 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.

Product quality control

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

Symbols

	Caution		Consult instructions for use
	Catalog number	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Use by		Temperature limitation
	Manufacturer		Serial Number
	Country and date of manufacturing		

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.

52-54 avenue du X Septembre, 2550 Luxembourg, Luxembourg

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the device. 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 1.0

Effective date: 24th November 2021

DeepChek® Assay

Whole Genome HBV Genotyping

Σ
24

User Guide

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 **184A24 – GTIN:** 05407007960606

Publication number: IFU_184A24_RUO_V1_R1

August 2023

Document control

Date	Device version	IFU version	Description of change
21/08/2023	A	1.1	• Change term “RT-PCR” by “PCR” • Changes in Application and Principle of the assay sections • Adapt the Workflow overview in figure 2 • Remove text “Wait until the thermal cycler has reached 95°C. Then place the PCR tubes in the thermal cycler” in step 5 of the PCR reaction
20/09/2022	A	1.0	• Document creation

Contents

Application

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.

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 Hepatitis B Virus in HBV specimens, including regions which harbor mutations described as sufficient, when present, to determine level of resistance to antiviral 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 antiviral drugs, specifically the Polymerase, the PreCoce, the Core, The PreS1/2 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 V1 (RUO)

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

	Master Mix 2X	H_2O	Nested Master Mix 2X	
	Frag 1	Frag 2	Frag 3	
	Nested Frag 1	Nested Frag 2	Nested Frag 3	

*Figure 1: Disposal of the assay components for **the DeepChek® Assay Whole Genome HBV Genotyping V1** (RUO)*

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 V1.x (RUO)***, 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 one or 2 mL of specimen and elute in the minimum volume required for your preferred extraction kit.

Workflow

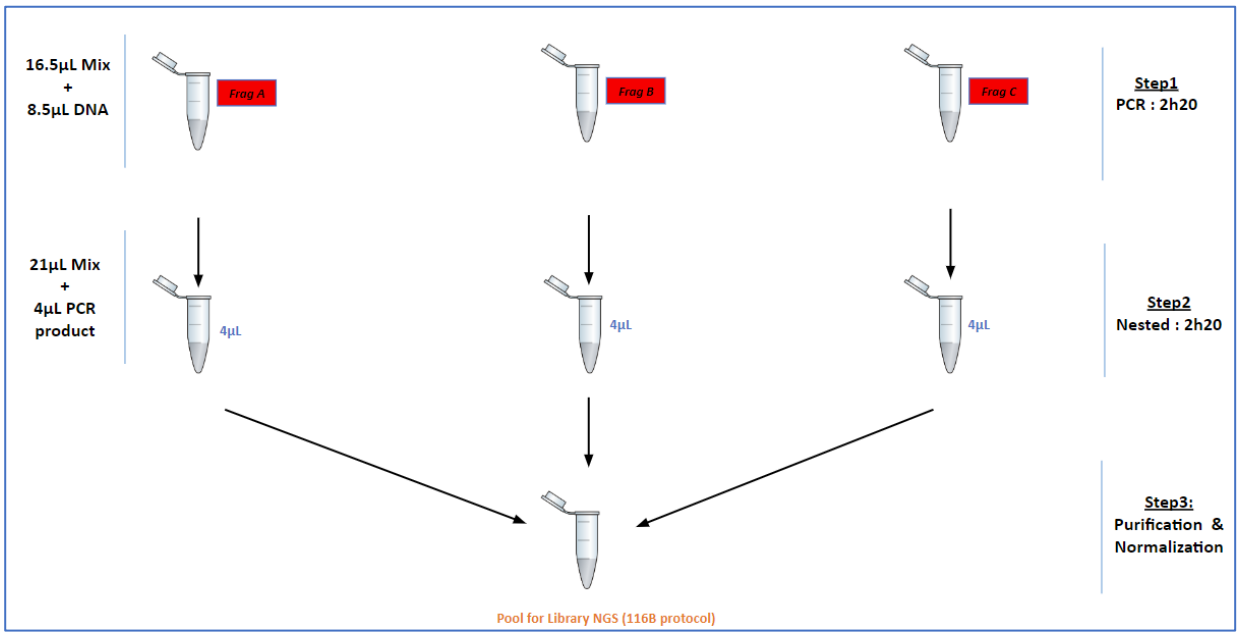


Figure 2: Workflow overview of the DeepChek Assay Whole Genome HBV Genotyping

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

Table 3: 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	∞

5. 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.
6. **[Recommended]** – Each PCR product 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. In that case, first use the DeepChek® Nested PCR reagents.

Note: expected amplicons size after the 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: Nested 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**.

Δ Safe Stopping Point: 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

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)). 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		
	Country and date of manufacturing		
	Distributor		

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

Advanced Biological Laboratories (ABL) S.A.
52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the device. 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
Effective date: 21st of August 2023

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.

REF **184A24**

Publication number: IFU_184A24_RUO_V1_R0

November 2022

Document control

Date	Device version	IFU version	Description of change
31/10/2022	A	V1.0	• Document creation

Contents

Application

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.

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

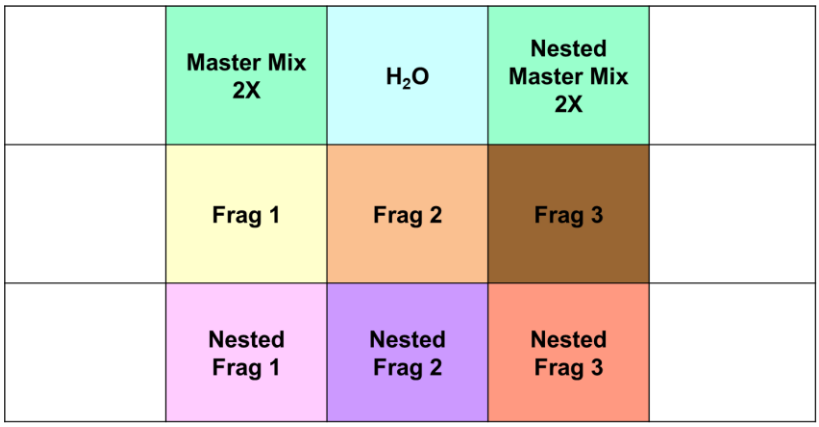

Figure 1: Disposal of the assay components for the DeepChek® Assay Whole Genome HBV Genotyping

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

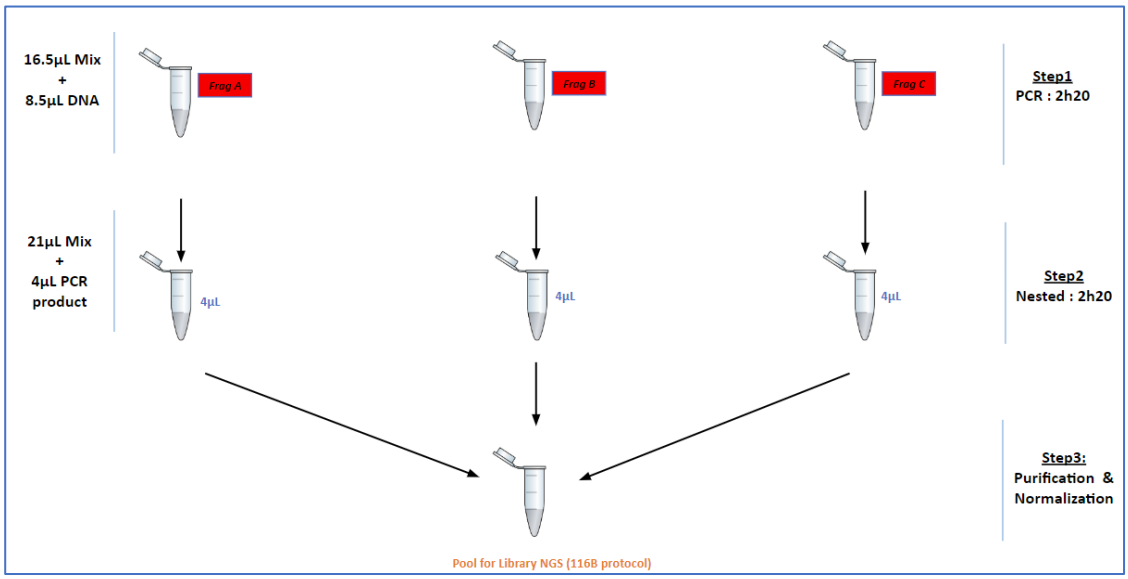

Figure 2: Workflow overview of the DeepChek® Assay Whole Genome HBV Genotyping

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

Advanced Biological Laboratories (ABL) S.A.
52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

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Version 1.0
Effective date: 17th of November 2022

DeepChek® Assay

Whole Genome HDV Genotyping

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User Guide

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 **199A24** **GTIN: 05407007961085**

Publication number: IFU_199A24_RUO_EN_V1_R1

July 2024

Document control

Date	Device version	IFU version	Description of change
2024/07/03	A	1.1	Correction of volumes reported on table 1
2023/10/12	A	1.0	Document creation

Contents

Application

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.

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** aims to amplify the complete genome of the Human Hepatitis delta virus in two PCR reactions. This nucleic acid amplification method screens the mutations in the small Human Hepatitis delta genome that has approximately 1700 nucleotides.

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of PCR and sequencing workflows.

Principles of the assay

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** is a reverse transcriptase-polymerase chain reaction test which includes primers, reverse and forward, designed to amplify HDV extracted RNA specimens.

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.

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** is performed on a PCR instrument.

Assay components

The ***DeepChek® Assay Whole Genome HDV Genotyping*** is provided in one model of 24 reactions (reference: 199A24).

Table 1: Volumes and storage conditions of the assay with reference ***199A24 V1 (RUO)***

Label	Volume for 24 Rxn. (nb. tube x volume)	Color cap	Storage
RT SuperMix	1 x 65 µL	Green	-25°C to - 15 °C
Master Mix HF	1 x 600 µL	Yellow	-25°C to - 15 °C
H_2O	1 x 500 µL	Blue	-25°C to - 15 °C
HDV-A1 FOR (10 µM)	1 x 35 µL	Brown	-25°C to - 15 °C
HDV-A2 REV (10 µM)	1 x 35 µL	Red	-25°C to - 15 °C
HDV-B1 FOR (10 µM)	1 x 35 µL	Pink	-25°C to - 15 °C
HDV-B2 REV (10 µM)	1 x 35 µL	Black	-25°C to - 15 °C

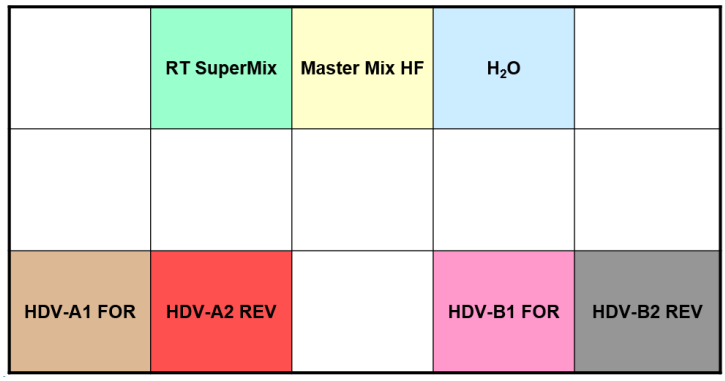


Figure 1: Disposal of the assay components for the assay with reference ***199A24 V1 (RUO)***

Reagent storage and handling

The ***DeepChek® Assay Whole Genome HDV Genotyping*** is shipped on 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
- 96-well plate cooler (Eppendorf / 22510509)
- 96-well PCR plates (Eppendorf / 951020303)
- Plate thermo seals (Thermo Scientific / AB-0558)
- Plate centrifuge
- 0.2 mL thin-wall 8 strips & domed caps (Thermo Scientific / AB-0266)
- 1.5 mL centrifuge tubes (Dot Scientific Inc. / RN1700-GST)
- Centrifuge tubes (see your specific centrifuge manual)
- Mini centrifuge (see your specific centrifuge manual)
- Microliter pipettes 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 and to the 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.
- 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 from the surface by wiping with 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 applicable regulations.
- Frequent cleaning of the wells of the PCR instrument thermos-blocks is recommended to prevent contamination.
- To avoid contamination, use separated and segregated working areas for extraction and PCR/sequencing preparation, respectively.
- 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

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 HDV RNA analysis, for the best representation of the viral quasispecies, it is recommended to extract **1 mL** of specimen for subsequent cDNA and amplicon generation and elute in the minimum volume required for your preferred extraction kit. Samples which are hemolyzed, icteric or lipemic can invalidate extraction.

The ***DeepChek® Assay Whole Genome HDV Genotyping*** will work with at least an extraction of 400 µL of specimen (i.e., plasma, serum, whole blood) specimens, to be eluted in 60 µL.

For specimens with low viral load, we recommend:

- 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

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

RT reaction setup

1. Thaw extracted template RNA, RT SuperMix, and RNase-free water and place them on ice. Load all the tubes into the centrifuge. Spin the tube at 11,000 g for 10 seconds. And then pipette up and down the mix several times before dispensing.
2. The master mix typically contains all the components required for RT except the template RNA. Prepare a volume of mix greater (n+1) than that required for the total number of reactions to be performed.
3. Vortex the master mix thoroughly and dispense 10 µL into PCR tubes.
4. Add 10 µL of RNA in the PCR tubes. Mix by pipetting the solution up and down a few times.

Table 2: Reaction components for RT.

Reagent	Volume / Reaction
RT SuperMix	2 µL
Extracted viral RNA template	10 µL
H_2O	8 µL

5. Program the thermal cycler according to the program in the next table.

Table 3: RT cycling program.

Cycle	Temperature (°C)	Time
1	25 °C	2 min
1	55 °C	10 min
1	95 °C	1 min
	4 °C	Hold

Note: Ramp rate shall be at 6°C/s.

PCR reaction setup

1. The master mix typically contains all the components required for PCR except the template cDNA. Prepare a volume of master mix greater (n+1) than that required for the total number of reactions to be performed. Prepare PCR WG Fragment 1 & 2 master mix according to next table and following instructions.
2. Vortex the master mix thoroughly and dispense **14** µL into PCR tubes.
3. Add **6** µL of cDNA from the RT step in the PCR tubes. Mix by pipetting the solution up and down a few times.

Table 4: Reaction components for PCR.

Reagent	Volume / Reaction
PCR WG Fragment 1	
Master Mix HF	10 µL
HDV-A1 FOR	1 µL
HDV-A2 REV	1 µL
H_2O	2 µL
PCR WG Fragment 2	
Master Mix HF	10 µL
HDV-B1 FOR	1 µL
HDV-B2 REV	1 µL
H_2O	2 µL

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

Table 5: PCR cycling program

Cycle	Temperature (°C)	Time
Enzyme activation	98 °C	30 sec
35 cycles	98 °C	10 sec
	65 °C	30 sec
	72 °C	30 sec
Final extension	72 °C	2 min
	4 °C	Hold

Note: Ramp rate shall be at 6°C/s.

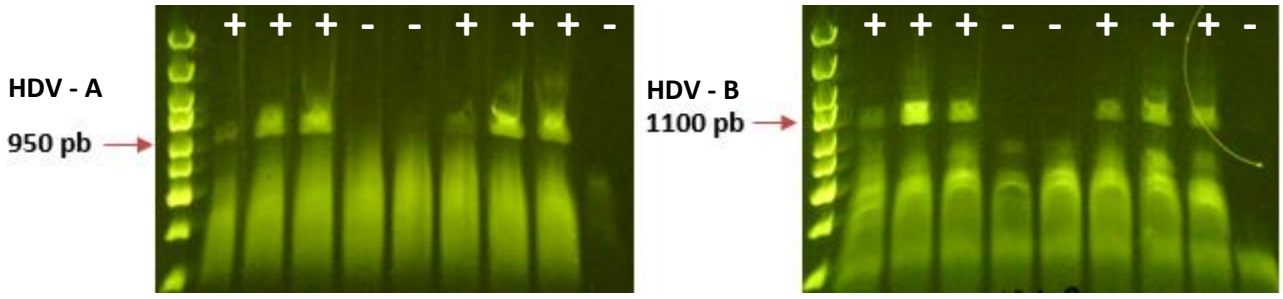
5. Start the program while PCR tubes are still on ice.
6. Once ready, place the PCR-tubes in the designated positions of the thermo block to run the amplification process.

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

7. PCR products shall be controlled through electrophoresis on a 1% agarose gel. Check the intensity of the signal. Even though 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, please read the troubleshooting section hereinafter.

Note: Primer pairs used in this assay produce two partially overlapping segments covering the entire HDV genome.

The expected amplicon sizes are **PCR 1 ≈ 950 bp (HDV-A)** and **PCR 2 ≈ 1100 bp (HDV-B).**



Troubleshooting guide

Use the following troubleshooting table to diagnose and solve problems. The troubleshooting recommendations assume that all the DeepChek® Assay reagents are stored according to the specifications and that the directions in this guide have been followed correctly.

	Comments and suggestions
Negative controls are positive	
Cross-contamination	Replace all critical reagents. Repeat the experiment with new reagents. Always handle samples, assay components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.
Absent or low signal in samples	
a. Poor DNA quality or inadequate concentration	Check your (capillary) gel electrophoresis preparation. You may dilute your DNA product according to your (capillary) gel electrophoresis procedure. Follow the instructions for use if using a capillary electrophoresis instrument. Rerun your (capillary) gel electrophoresis.
b. Sample prepared too long before analysis leading to evaporation	Check the volume of the RT-PCR product in the PCR tube/plate. Repeat the PCR of the affected sample target.
c. Poor agarose gel conditions or capillary electrophoresis instrument reagents used are incorrect or unproper	Use new (capillary) gel electrophoresis reagents. Rerun your (capillary) gel electrophoresis.

Comments and suggestions	
No band signal for one or few samples	
a. The PCR didn't work	Take the corresponding PCR product. Run the (capillary) gel electrophoresis. If still no band signal, start again the whole protocol.
b. Degraded RNA isolation from initial blood sample	Check again the viral load of the HDV test. A too low RNA concentration (<10 fg) may result in a negative result. If the RNA concentration is > 10 fg then you may repeat the whole protocol or improve the conditions of the specimen (e.g., ultra-centrifugation, RNA concentration) and do a new RNA isolation on fresh or frozen specimen and start again the whole protocol. Contact our technical support.
c. Limitation variant	If you obtain repeatedly negative results, and other potential error sources have been ruled out, the sample could contain a variant that contains mutations in the primer binding site. Contact our technical support.

Post PCR

Next Generation Sequencing

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

While performing the verification studies, we used the Illumina iSeq 100 Sequencing System (catalog #20021535), the Illumina MiSeq Sequencing System (catalog #SY-410-1003) and the following combination of reagents: ABL ***DeepChek® NGS Library preparation*** (catalog #116BX, 24 or 48 or 96 tests), ABL ***DeepChek® Assay Adapters*** (catalog #124BX, 1-24, 1-48 or 1-96) , Illumina iSeq 100 Reagent (catalog # 20021533, 300 cycles) and Illumina MiSeq Reagent (catalog #MS-102-2003, 500 cycles)

Details available on demand for other NGS analyzers and NGS reagents and technology, please contact our technical support.

Downstream NGS data analysis

The sequencing raw data can then be uploaded in a specific downstream software or a generic bioinformatics tool for HDV genome analysis and results. This software can be classified a standalone medical device according to its intended use. 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

REF	Catalog number	SN	Serial Number
	Use by	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Manufacturer		Country of manufacturing
	Distributor		

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

Advanced Biological Laboratories (ABL) S.A.
52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

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Version 1.1

Effective date: 3rd of July 2024

DeepChek® Assay

Whole Genome HDV Genotyping

Σ
24

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.

REF **199A24**

Publication number: IFU_199A24_RUO_EN_V1_R0

October 2023

Document control

Date	Device version	IFU version	Description of change
2023/10/12	A	1.0	Document creation

Contents

Application

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.

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** aims to amplify the complete genome of the Human Hepatitis delta virus in two PCR reactions. This nucleic acid amplification method screens the mutations in the small Human Hepatitis delta genome that has approximately 1700 nucleotides.

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of PCR and sequencing workflows.

Principles of the assay

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** is a reverse transcriptase-polymerase chain reaction test which includes primers, reverse and forward, designed to amplify HDV extracted RNA specimens.

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.

The ***DeepChek® Assay Whole Genome HDV Genotyping (RUO)*** is performed on a PCR instrument.

Assay components

The ***DeepChek® Assay Whole Genome HDV Genotyping*** is provided in one model of 24 reactions (reference: 199A24).

Table 1: Volumes and storage conditions of the assay with reference ***199A24 V1 (RUO)***

Label	Volume for 24 tests	Color cap	Storage
RT SuperMix	58 µL	1	-25°C to - 15 °C
Master Mix HF	580 µL	1	-25°C to - 15 °C
H_2O	348 µL	1	-25°C to - 15 °C
HDV-A1 FOR (10 µM)	29 µL	1	-25°C to - 15 °C
HDV-A2 REV (10 µM)	29 µL	1	-25°C to - 15 °C
HDV-B1 FOR (10 µM)	29 µL	1	-25°C to - 15 °C
HDV-B2 REV (10 µM)	29 µL	1	-25°C to - 15 °C

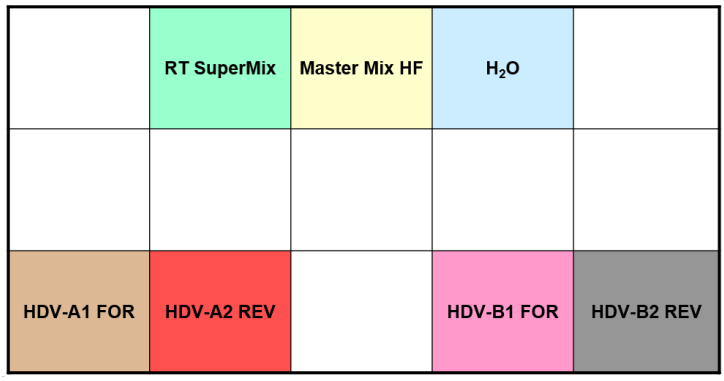


Figure 1: Disposal of the assay components for the assay with reference ***199A24 V1 (RUO)***

Reagent storage and handling

The ***DeepChek® Assay Whole Genome HDV Genotyping*** is shipped on 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
- 96-well plate cooler (Eppendorf / 22510509)
- 96-well PCR plates (Eppendorf / 951020303)
- Plate thermo seals (Thermo Scientific / AB-0558)

- Plate centrifuge
- 0.2 mL thin-wall 8 strips & domed caps (Thermo Scientific / AB-0266)
- 1.5 mL centrifuge tubes (Dot Scientific Inc. / RN1700-GST)
- Centrifuge tubes (see your specific centrifuge manual)
- Mini centrifuge (see your specific centrifuge manual)
- Microliter pipettes 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 and to the 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.
- 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 from the surface by wiping with 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 applicable regulations.
- Frequent cleaning of the wells of the PCR instrument thermos-blocks is recommended to prevent contamination.
- To avoid contamination, use separated and segregated working areas for extraction and PCR/sequencing preparation, respectively.
- 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

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 HDV RNA analysis, for the best representation of the viral quasispecies, it is recommended to extract **1 mL** of specimen for subsequent cDNA and amplicon generation and elute in the minimum volume required for your preferred extraction kit. Samples which are hemolyzed, icteric or lipemic can invalidate extraction.

The ***DeepChek® Assay Whole Genome HDV Genotyping*** will work with at least an extraction of 400 µL of specimen (i.e., plasma, serum, whole blood) specimens, to be eluted in 60 µL.

For specimens with low viral load, we recommend:

- 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

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

RT reaction setup

1. Thaw extracted template RNA, RT SuperMix, and RNase-free water and place them on ice. Load all the tubes into the centrifuge. Spin the tube at 11,000 g for 10 seconds. And then pipette up and down the mix several times before dispensing.
2. The master mix typically contains all the components required for RT except the template RNA. Prepare a volume of mix greater (n+1) than that required for the total number of reactions to be performed.
3. Vortex the master mix thoroughly and dispense 10 µL into PCR tubes.
4. Add 10 µL of RNA in the PCR tubes. Mix by pipetting the solution up and down a few times.

Table 2: Reaction components for RT.

Reagent	Volume / Reaction
RT SuperMix	2 µL
Extracted viral RNA template	10 µL
H_2O	8 µL

5. Program the thermal cycler according to the program in the next table.

Table 3: RT cycling program.

Cycle	Temperature (°C)	Time
1	25 °C	2 min
1	55 °C	10 min
1	95 °C	1 min
	4 °C	Hold

Note: Ramp rate shall be at 6°C/s.

PCR reaction setup

1. The master mix typically contains all the components required for PCR except the template cDNA. Prepare a volume of master mix greater (n+1) than that required for the total number of reactions to be performed. Prepare PCR WG Fragment 1 & 2 master mix according to next table and following instructions.
2. Vortex the master mix thoroughly and dispense **14** µL into PCR tubes.
3. Add **6** µL of cDNA from the RT step in the PCR tubes. Mix by pipetting the solution up and down a few times.

Table 4: Reaction components for PCR.

Reagent	Volume / Reaction
PCR WG Fragment 1	
Master Mix HF	10 µL
HDV-A1 FOR	1 µL
HDV-A2 REV	1 µL
H_2O	2 µL
PCR WG Fragment 2	
Master Mix HF	10 µL
HDV-B1 FOR	1 µL
HDV-B2 REV	1 µL
H_2O	2 µL

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

Table 5: PCR cycling program

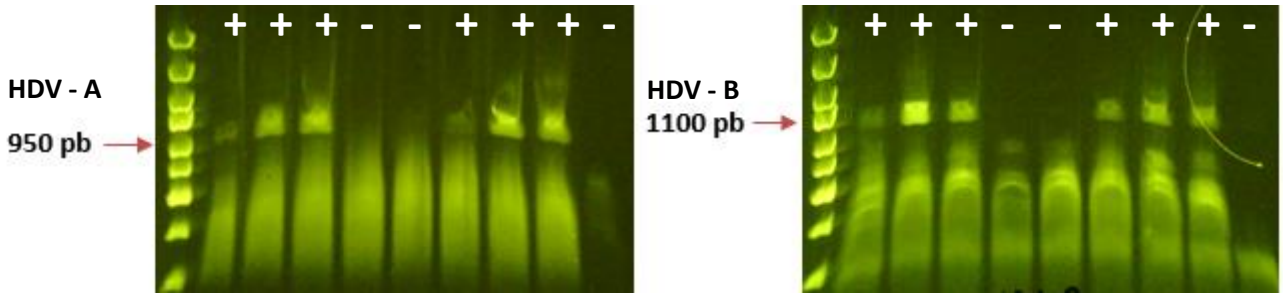
Cycle	Temperature (°C)	Time
Enzyme activation	98 °C	30 sec
35 cycles	98 °C	10 sec
	65 °C	30 sec
	72 °C	30 sec
Final extension	72 °C	2 min
	4 °C	Hold

Note: Ramp rate shall be at 6°C/s.

5. Start the program while PCR tubes are still on ice.
6. Once ready, place the PCR-tubes in the designated positions of the thermo block to run the amplification process.
   Δ Safe Stopping Point: After amplification, specimens can be stored overnight at 2–10°C, or at -20°C for long-term storage.
7. PCR products shall be controlled through electrophoresis on a 1% agarose gel. Check the intensity of the signal. Even though 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, please read the troubleshooting section hereinafter.

Note: Primer pairs used in this assay produce two partially overlapping segments covering the entire HDV genome.

The expected amplicon sizes are **PCR 1 ≈ 950 bp (HDV-A)** and **PCR 2 ≈ 1100 bp (HDV-B).**



Troubleshooting guide

Use the following troubleshooting table to diagnose and solve problems. The troubleshooting recommendations assume that all the DeepChek® Assay reagents are stored according to the specifications and that the directions in this guide have been followed correctly.

	Comments and suggestions
Negative controls are positive	
Cross-contamination	Replace all critical reagents. Repeat the experiment with new reagents. Always handle samples, assay components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.
Absent or low signal in samples	
a. Poor DNA quality or inadequate concentration	Check your (capillary) gel electrophoresis preparation. You may dilute your DNA product according to your (capillary) gel electrophoresis procedure. Follow the instructions for use if using a capillary electrophoresis instrument. Rerun your (capillary) gel electrophoresis.
b. Sample prepared too long before analysis leading to evaporation	Check the volume of the RT-PCR product in the PCR tube/plate. Repeat the PCR of the affected sample target.
c. Poor agarose gel conditions or capillary electrophoresis instrument reagents used are incorrect or unproper	Use new (capillary) gel electrophoresis reagents. Rerun your (capillary) gel electrophoresis.
No band signal for one or few samples	
a. The PCR didn't work	Take the corresponding PCR product. Run the (capillary) gel electrophoresis. If still no band signal, start again the whole protocol.
b. Degraded RNA isolation from initial blood sample	Check again the viral load of the HDV test. A too low RNA concentration (<10 fg) may result in a negative result. If the RNA concentration is > 10 fg then you may repeat the whole protocol or improve the conditions of the specimen (e.g., ultra-centrifugation, RNA concentration) and do a new RNA isolation on fresh or frozen specimen and start again the whole protocol. Contact our technical support.
c. Limitation variant	If you obtain repeatedly negative results, and other potential error sources have been ruled out, the sample could contain a variant that contains mutations in the primer binding site. Contact our technical support.

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Next Generation Sequencing

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Downstream NGS data analysis

The sequencing raw data can then be uploaded in a specific downstream software or a generic bioinformatics tool for HDV genome analysis and results. This software can be classified a standalone medical device according to its intended use. 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		Negative control
	Catalog number		Positive control
	Use by		Temperature limitation
	Manufacturer		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: 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

Advanced Biological Laboratories (ABL) S.A.
52-54 avenue du X Septembre
2550 Luxembourg, Luxembourg

AdvancedDx Biological Laboratories USA Inc.
5-7 Perry Way, Unit 15 Newburyport, MA 01950, USA

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Version 1.0

Effective date: 12th October 2023

1 Components

- EXTRAS MANUAL -

1.1 Kit contents

	NucleoSpin® Blood		
REF	10 preps 740951.10	50 preps 740951.50	250 preps 740951.250
Lysis Buffer B3	10 mL	15 mL	60 mL
Wash Buffer BW	6 mL	30 mL	150 mL
Wash Buffer B5 (Concentrate)*	6 mL	12 mL	50 mL
Elution Buffer BE**	13 mL	13 mL	60 mL
Proteinase K (lyophilized)*	6 mg	30 mg	2 × 75 mg
Proteinase Buffer PB	1.8 mL	1.8 mL	8 mL
NucleoSpin® Blood Columns (red rings – plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	20	100	500
User manual	1	1	1

PAG 1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1 Components - EXTRAS MANUAL -

1.1 Kit contents

	NucleoSpin® RNA Virus		
REF	10 preps 740956.10	50 preps 740956.50	250 preps 740956.250
Lysis Buffer RAV1	10 mL	35 mL	5 × 35 mL
Wash Buffer RAW	6 mL	30 mL	150 mL
Wash Buffer RAV3 (Concentrate)*	6 mL	12 mL	50 mL
RNase-free H_2O	13 mL	13 mL	30 mL
Elution Buffer RE**	13 mL	13 mL	30 mL
Carrier RNA (lyophilized)	300 µg	1 mg	5 × 1 mg
NucleoSpin® RNA Virus Columns (dark blue rings, plus Collection Tubes)	10	50	250
Collection Tubes (2 mL)	30	150	750
User manual	1	1	1

PAG 1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer RE: 5 mM Tris/HCl, pH 8.5

NucleoSpin Blood, Mini kit for DNA from blood

Product configuration

Selling unit

Please select

Do you have any questions?
Please contact us.

Select variant first

*taxes and shipping not included

Item number: 740951.50
Package unit 50 Preps

NucleoSpin Blood is a spin column based kit for manual use. It is intended for the isolation of DNA from whole blood, stabilized with EDTA, citrate, or heparin, serum, plasma, and other body fluids. Besides spin columns and buffers it contains Proteinae K for efficient blood lysis.

Application	Isolation of DNA
Selling unit	10 Prep(s), 50 Prep(s), 250 Prep(s)
Target	DNA
CE certified	No, research use only
Technology	Silica membrane technology
Brand	NucleoSpin
Format	Mini prep
Handling	Centrifugation
Automated use	No
Sample material	Blood, Body fluids, Buffy coat, Cells, Plasma, Platelets, Serum
Sample amount	5–200 µL whole blood (human or animal, fresh or frozen, treated with citrate, EDTA, heparin, or CPDA), buffy coat, platelets, body fluids, serum, plasma, < 5 x 10^6 human/animal cells cultured cells
Fragment size	200 bp–approx. 50 kbp
Typical yield	4–6 µg
Typical concentration	40–100 ng/µL
Theoretical binding capacity	60 µg
Typical purity A260/A280	1.6–1.9
Elution volume	60–200 µL
Preparation time	30 min/prep
Typical downstream application	enzymatic reactions, Next Generation Sequencing, PCR, Southern blotting
Storage temperature	15–25 °C / 59–77 °F
Shelf life (from production)	27 Month(s)

NucleoSpin RNA Virus, Mini kit for viral RNA from cell-free fluids

Product configuration

Selling unit

Please select

Do you have any questions?
Please contact us.

Select variant first

*taxes and shipping not included

Item number: 740956.50
Package unit 50 Preps

NucleoSpin Virus is a spin column based kit for manual use. Is is intended for the isolation of viral RNA from cell-free fluids, serum, and plasma. Besides spin columns and buffer it contains carrier RNA to enable high RNA recovery rates from low titer viruses.

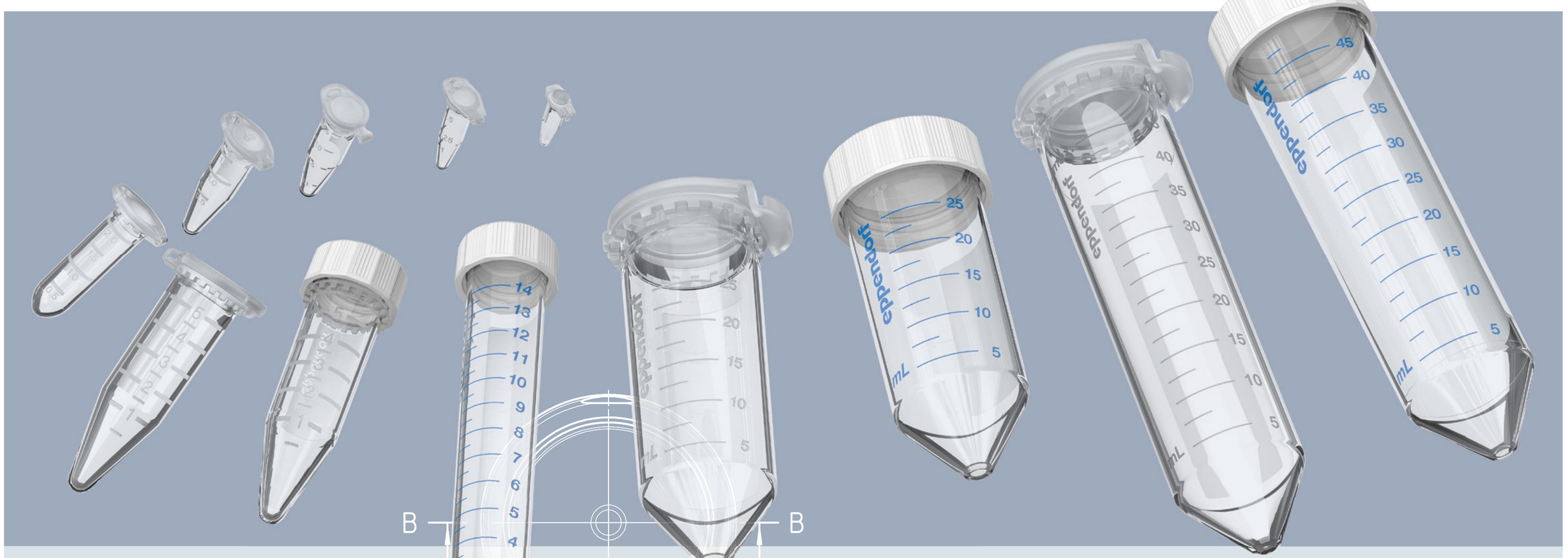
Selling unit	10 Prep(s), 50 Prep(s), 250 Prep(s)
Application	Isolation of viral nucleic acids
Target	Viral RNA and DNA
CE certified	No, research use only
Technology	Silica membrane technology
Brand	NucleoSpin
Format	Mini prep
Handling	Centrifugation
Automated use	No
Sample material	Cell-free biological fluid, Plasma, Serum
Sample amount	< 150 µL
Fragment size	100 bp–approx. 50 kbp
Theoretical binding capacity	40 µg
Elution volume	50 µL
Preparation time	30 min/4–6 preps
Typical downstream application	enzymatic reactions, RT-PCR
Storage temperature	15–25 °C / 59–77 °F
Shelf life (from production)	27 Month(s)
Hazardous material	Yes

Related products +

eppendorf

Absolute Tubes

Your best choice to care for your sample:
Eppendorf Tubes®



It’s Your Sample

Disposable plasticware is used routinely in most life science laboratories. To achieve the highest level of reliability and consistency for your experiments, Eppendorf has optimized source materials and processes for the production of Eppendorf Tubes® to minimize the risk of interference. The effects from chemical substances such as slip agents, plasticizers or biocides leaching out of plastic consumables are still frequently underestimated in the majority of life science applications. However, increasing scientific evidence shows that this heterogeneous group of chemicals may significantly affect experiments and pose a likely source of error in various assay systems. They slow down evaporation, skew absorbance readings and lead to erroneous DNA quantification. Some of these slip agents have also been shown to negatively affect the outcome of biological tests like enzyme activity or receptor-binding assays.

Eppendorf Tubes®

> Made of the highest quality, either virgin and bio-circular polypropylene (PP), free of biocides, plasticizers and latex
> Produced with optimized, highly polished molds without the use of slip agents like oleamide, erucamide or stearamide
> Designed and manufactured under ISO 13485 and ISO 9001 certified Management System
> Full traceability is provided for each product back to material lot

Purity tailored to your applicational needs

Eppendorf has set standards in consumable purity levels. In addition to the high standard »Eppendorf Quality«, four additional purity grades are specifically tailored to the applications in which Eppendorf Tubes are used – Sterile (incl. pyrogen-free, RNase-free, DNase-free, human and bacterial DNA-free), PCR clean, Forensic DNA Grade and Biopur®. Tubes with these purity grades are tested by an independent, external laboratory for compliance. The lot-specific certificates issued by this laboratory may be downloaded at **www.eppendorf.com/certificates**

Eppendorf PCR Tubes

Original Eppendorf PCR tubes are manufactured according to the highest Eppendorf Quality standards. These thin-walled polypropylene tubes ensure efficient and homogeneous heat transfer. They are easy to open, but provide tight sealing to prevent evaporation during PCR. Efficient heat transfer to the sample is ensured, thanks to their thin, even wall thickness and smooth wall surface.

Eppendorf PCR Tubes, 0.2 mL
> Contamination shield on hinged lid
> Defined lid position due to special hinge
> High transparency even at the base of the tube
> Etched lid for labeling
> For use with thermal cyclers with 0.2 mL block format
> Also available in 8-tube strip format
> Lot specific certified free from human DNA, DNase, RNase and PCR inhibitors*

Eppendorf PCR Tubes, 0.5 mL
> Etched lid for labeling
> Tight seal but easy to open
> For use with all thermal cyclers with 0.5 mL block format
> Lot specific certified free from human DNA, DNase, RNase and PCR inhibitors*

Eppendorf PCR Tube Strips
> 8 reaction tubes in strip-format – ideal for small sample volumes
> Easily adaptable for automation
> Sealable using flat or domed strip-lids
> Lot specific certified free from human DNA, DNase, RNase and PCR inhibitors*

Cap Strips
> Strips with eight microcaps for PCR tube strips
> Easy and rapid sealing of Eppendorf PCR tube strips
> Flat Cap Strips are suitable for real-time PCR
> Autoclavable (121 °C, 20 min)

Masterclear® Cap Strips and *real-time* PCR Tube Strips
> White wells for better reflection
> High mechanical stability
> Extremely thin walls for optimal heat transfer
> Cap strips with inverted dome to reduce volume of the tubes
> Cap strips optimized for maximum light transmission

*Download of certificates on www.eppendorf.com/certificates

Eppendorf SafeCode System

»Stored vessels must be labeled« – naturally, every lab member agrees. In reality you always find some (or even more) vessels in your freezer without any labeling or with labeling resembling hieroglyphs. In many labs, there is a second rule: label-free vessels are disposed of as soon as they are found.

Proper labeling is recommended to make reading easy and as reliable as possible for everyone. Printed labels on vessels can contain either plain writing, barcode, or both. Smart labeling of your high-value samples is crucial for safe identification and ultimately for safe results. Manage your (barcoded) samples with sample management software like eLabNext.

Relax – Your Samples Are Safe

As a scientist, you own hundreds of samples; samples that are the results of years of hard work; samples of high value. When storing these, it is vital to keep them safe and and know their ID.

The SafeCode system is based on a multi-level-coding to enable safe sample identification: QR code and human readable code.
> Pre-labeled off-the-shelf consumables for immediate use
> Reliable long-term labels for safe sample ID
> Combine all experimental data with relevant information about the vessel for convenient documentation
> Available as cryostorage vials from 0.5 mL–4.0 mL for cold storage
> Available as 5 mL, 15 mL, and 50 mL tubes, combining the well-known Eppendorf tube benefits with digital approaches

The Eppendorf SafeCode family:
For modern digital sample management with high sample safety, management, and tracking needs.

> www.eppendorf.com/SafeCode

APPLICATION NOTE No. 266

Eppendorf PCR Consumables – Compatibility Guide for PCR and qPCR Cyclers

Natascha Weiß[1], Rafal Grzeskowiak[1], Sandrine Hamels[2], Beate Riekens[1]
[1]Eppendorf AG, Hamburg, Germany; [2]Eppendorf Array Technologies SA, Namur, Belgium

Abstract

One of the key issues regarding PCR consumables performance (plates, stripes and respective sealing options) is their compatibility with several cycler systems available on the market. In this Application Note, Eppendorf PCR/qPCR Consumables were tested for compatibility with the most important cycler brands via a physical fit test with the cycler blocks as well as through exemplary PCR and qPCR assays for some instruments.

Introduction

PCR and qPCR consumables vary in numerous parameters. Among them material properties and quality, well/skirt dimensions, well thickness and surface characteristics play the most important role. These differences together with various sealing options lead to often substantial variances when changing from one consumable brand to another and can influence overall PCR/qPCR assay performance.

It is therefore of much importance to identify whether a given consumable is compatible with a given PCR/qPCR platform. Here we present an overview of the Eppendorf twin.tec® PCR Plates, Eppendorf PCR Tube Strips as well as Eppendorf Cap Strips and their compatibility with the major cycler brands currently on the market.

Materials and Methods

Compatibility with standard PCR and qPCR devices
In most cases, the compatibility of the Eppendorf PCR Consumables was determined by their fit in the corresponding cycler block in combination with the closure of the cycler lid. Some systems were also tested for their performance in an PCR or qPCR assay.
Of course, these experiments are not representative. Therefore, it will always be necessary for each user to test the consumables in their own application.

PCR assay: Amplification of gDNA (Promega®) in a PCR. After the PCR assay the samples were separated by agarose gel electrophoresis (E-Gel®, Invitrogen®) and documented. Compatibility and assay performance was evaluated by presence and quality of PCR products on the gel (data not shown).

qPCR assay: For the qPCR assay performance, a standard assay with lambda DNA (Promega) and KAPA™ SYBR® Fast qPCR Mastermix (KAPA Biosystems®) was used. Three independent qPCR assays over six concentration logs of the lambda DNA template (10^1-10^6) and 3 replicates for each concentration were performed. Compatibility was evaluated based on following qPCR reaction parameters: C_t mean, C_t SD, CV, melting curves analysis (data not presented).

Results

Table 1: Compatibility with Standard PCR cyclers

PCR Instrument	twin.tec PCR Plate 96						twin.tec PCR Plate 384	Tube Strips		Cap Strips	
	unskirted, divisible (standard profile)	**unskirted** (standard profile)	**unskirted, low profile, divisible**	**unskirted, low profile**	**semi-skirted** (standard profile)	**skirted** (low profile)		**PCR Tube Strips 0.2 mL**	**PCR Tube Strips 0.1 mL**	**Cap Strips, domed**	**Cap Strips, flat**
ABI® GeneAmp® 2700/2720	+	+	–	–	+	–	–	+*	–	+	+
ABI GeneAmp 9600	+	+	–	–	+	–	–	+*	–	+	+
ABI GeneAmp 9700 (96-well)	+	+	–	–	+	–	–	+*	–	+	+
ABI GeneAmp 9700 (384-well)	–	–	–	–	–	–	+	–	–	–	–
ABI GeneAmp 9800 Fast	–	–	+	+	–	–	–	–	+	+	+
ABI MiniAmp®	+	+	–	–	+	–	–	+	–	+	+
ABI SimpliAmp®	+	+	–	–	+	–	–	+	–	+	+
ABI VeritiPro Thermal Cycler	+	+	–	–	+	–	–	+	–	+	+
ABI ProFlex® (96-well)	+	+	–	–	+	–	–	+	–	+	+
ABI Veriti® 96-well	+	+	–	–	+	–	–	+	–	+	+
ABI Veriti 96-well Fast	–	–	+	+	–	–	–	–	+	+	+
ABI Veriti 384-well	–	–	–	–	–	–	+	–	–	–	–
Agilent® Surecycler® 8800 (96-well)	+	+	–	–	+	–	–	+	–	+	+
Analytic Jena Alpha SC	–	–	+	+	–	+	–	–	+	+	+
Apollo ATC-201	+	+	+	+	+	+	–	+	+	+	+
Bioer Gene Explorer (96-well)	+	+	n.d.	n.d.	+	–	–	+	n.d.	+	+
Biometra® Tadvanced (96 /96 G / 96 S / 96 SG)	+	+	+	+	+	+	–	+	+	+	+
Biometra Tadvanced (384-well)	–	–	–	–	–	–	+	–	–	–	–
Biometra Tgradient 96	+	+	+	+	+	+	–	+	+	+	+
Biometra TOne	+	+	+	+	+	+	–	+	+	+	+
Biometra Tpersonal (combi block)	+	–	–	–	–	–	–	+	–	+	+
Biometra Tprofessional (96, 96 Gradient)	+	+	+	+	+	+	–	+	+	+	+
Biometra TRIO (combi block)	+	–	–	–	–	–	–	+	–	+	+
Biometra Uno	+	+	+	+	+	+	–	+	+	+	+
Bioer GeneExplorer 96	+	+	–	–	+	–	–	+	–	+	+
BIONEER® AllInOneCycler™ (96-well)**	+	+	–	–	+	–	–	+	–	+	+
Bio-Rad® C1000, C1000 Touch, S1000 (96-well)	+	+	+	+	+	+	–	+	+	+	+
Bio-Rad C1000, C1000 Touch, S1000 (384-well)	–	–	–	–	–	–	+	–	–	–	–
Bio-Rad iCycler (96-well)	+	+	–	–	+	–	–	+	–	+	+
Bio-Rad MyCycler	+	+	–	–	+	–	–	+	–	+	+
Bio-Rad PTC 200 (96-well)	+	+	+	+	+	+	–	+	+	+	+
Bio-Rad PTC Tempo Thermal Cycler 96-Well	+	+	+	+	+	+	–	+	–	+	+
Bio-Rad T100	+	+	–	–	+	–	–	+	–	+	+
G-Storm® GS1 (96-well)	+	+	+	+	+	+	–	+	+	+	+
HiMedia® Prima-96	+	+	n.d.	n.d.	+	–	–	+	n.d.	+	+
peqLab® peqSTAR® 96X	+	+	+	+	+	+	–	+	+	+	
peqLab® peqSTAR® XS32	–	–	–	–	–	–	–	+	–	–	–
TaKaRa® Dice TP600	+	+	+	+	n.d.	–	–	n.d.	n.d.	n.d.	n.d.
Techne® Touchgene (96-well)	+	+	+	+	+	+	–	+	+	+	+
Techne TC-412 (96-well)	+	+	+	+	+	+	–	+	+	+	+
Techne TC-PLUS (96-well)	+	+	+	+	+	+	–	–	+	–	+
VWR® Collection UNO^{96}	+	+	+	+	+	+	–	+	+	+	+
VWR® XT 96	+	+	+	–	+	–	–	+	–	+	+

(+): compatible, (-): not compatible, (n.d.): no data
*with ABI adapter, Standard accessory included with the respective cycler unit
**Cannot be used with low profile tubes or plates

Table 2: Compatibility with qPCR cyclers

qPCR Instrument	twin.tec PCR Plate 96						twin.tec PCR Plate 384	Tube Strips		Cap Strips	
	unskirted, divisible (standard profile)	**unskirted** (standard profile)	**unskirted, low profile, divisible**	**unskirted, low profile**	**semi-skirted** (standard profile)	**skirted** (low profile)		**PCR Tube Strips 0.2 mL**	**PCR Tube Strips 0.1 mL**	**Cap Strips, domed**	**Cap Strips, flat****
ABI 7300, 7500 Real-time PCR system	+	+	–	–	–	–	–	–	–	–	+
ABI 7500 Fast Real-time PCR system	–	–	+	+	–	–	–	–	+	–	+
ABI 7900HT Real-Time PCR System (Standard 96-well block)	+	+	–	–	–	–	–	–	–	–	+
ABI 7900HT Real-Time PCR System (Fast 96-well block)	–	–	+	+	–	–	–	–	+	–	+
ABI 7900HT Real-Time PCR System (384-well block)	–	–	–	–	–	–	+	–	–	–	–
ABI QuantStudio® 3, 5, 6, 7, 12 K Real-time PCR System (96-well 0.2 mL block)	+	+	–	–	+	–	–	–	–	–	+
ABI QuantStudio 3, 5, 6, 7, 12 K Real-time PCR System (96-well 0.1 mL block)	–	–	+	+	–	–	–	–	+	–	+
ABI QuantStudio 5, 6, 7, 12 K Real-time PCR System (384-well block)	–	–	–	–	–	–	+	–	–	–	–
ABI StepOnePlus®	–	–	+	+	–	–	–	–	+	–	+
Agilent Mx3000P®/Mx3005P® qPCR System	+	+	–	–	–	–	–	–	–	–	+
Agilent AriaMx® qPCR System	–	–	+	+	–	+	–	–	+	–	+
Analytik Jena® QTower^3 (G, touch)	n.d.	n.d.	+	+	n.d.	n.d.	–	–	n.d.	–	n.d.
Analytik Jena QTower^3 84	–	–	–	–	–	–	+	–	–	–	–
Bio-Rad CFX96 Touch™	–	–	+	+	–	+	–	–	+	–	+
Bio-Rad CFX96 Touch Deepwell™	+	+	+	+	+	+	–	–	+	–	+
Bio-Rad CFX384 Touch™	–	–	–	–	–	–	+	–	–	–	–
Bio-Rad iQ, iQ-5, myiQ PCR System	+	+	–	–	+	–	–	–	–	–	+
KogeneBiotech PowerAmp96™	+	+	–	–	–	–	–	–	–	–	+
Roche® LightCycler® 96	–	–	+	+	–	–	–	–	+	–	+
Roche LightCycler 480 (96-well block)	–	–	–	+*	–	–	–	–	+**	–	+
Roche LightCycler 480 (384-well block)	–	–	–	–	–	–	–	–	–	–	–

(+): compatible, (-): not compatible, (n.d.): no data

Note: Eppendorf twin.tec PCR Plates and Eppendorf Tube Strips are also available as real-time PCR variants with white wells. These exhibit a higher signal intensity during the reaction. The Eppendorf Tube Strips 0.2 mL with the attached domed caps as well as the domed Eppendorf Cap Strips fit into many of the real-time cyclers listed here, but are not suitable for qPCR where detection is performed from the top. Therefore, these products are marked as "not compatible".
* With Eppendorf twin.tec Adapter for LC480: Cat. #0030 133.412,
** With LightCycler 8-Tube Strip Adapter Plate (Roche Cat. #06612598001)
***Eppendorf Cap Strips, flat are generally suitable for real-time PCR. However, depending on the instrument, experimental setup, and/or reagents, using standard caps might interfere with signal detection. For optimal performance, use Eppendorf Masterclear Cap Strips with their inverted dome optimized for maximum light transmission.

Conclusion

We present here a comprehensive overview of the compatibility of Eppendorf PCR/qPCR consumables on the major cycler brands currently on the market. The results show broad range of compatibility of Eppendorf consumables on the main cycler platforms.

Ordering information

Description	Order no. International	Order no. North America
Eppendorf twin.tec® PCR Plates 96, skirted	0030 128 648	951020401
Eppendorf twin.tec® PCR Plates 96, semi-skirted	0030 128 575	951020303
Eppendorf twin.tec® PCR Plates 96, unskirted	0030 133 366	0030133366
Eppendorf twin.tec® PCR Plates 96, unskirted, divisible	0030 133 374	0030133374
Eppendorf twin.tec® PCR Plates 96, unskirted, low profile	0030 133 307	0030133307
Eppendorf twin.tec® PCR Plates 96, unskirted, low profile, divisible	0030 133 358	0030133358
Eppendorf twin.tec® PCR Plate 384	0030 128 508	951020702
Eppendorf twin.tec® *real-time* PCR Plates 96, skirted	0030 132 513	951022015
Eppendorf twin.tec® *real-time* PCR Plates 96, semi-skirted	0030 132 548	951022055
Eppendorf twin.tec® *real-time* PCR Plates 96, unskirted, low profile	0030 132 700	0030132700
Eppendorf twin.tec® *microbiology* PCR Plates 96, skirted	0030 129 300	0030129300
Eppendorf twin.tec® *microbiology* PCR Plates 96, semi-skirted	0030 129 326	0030129326
Eppendorf twin.tec® *microbiology* PCR Plates 384	0030 129 342	0030129342
PCR Tubes Strips 0.1 mL without caps	0030 124 804	0030124804
PCR Tubes Strips 0.2 mL	0030 124 359	951010022
real-time PCR Tubes Strips 0.1 mL without caps	0030 132 882	951022102
Cap Strips, flat	0030 124 847	0030124847
Cap Strips, domed	0030 124 839	0030124839
Masterclear® Cap Strips	0030 132 874	951022089
Masterclear® *real-time* PCR Film, adhesive	0030 132 947	0030132947
PCR Foil, adhesive	0030 127 790	0030127790
PCR Film, adhesive	0030 127 781	0030127781
Heat Sealing Foil	0030 127 854	0030127854
Heat Sealing Film	0030 127 838	0030127838

Note: Order numbers for twin.tec PCR Plates and twin.tec *real-time* PCR Plates correspond to colorless and white skirts respectively. There are other skirt colors as well as more consumables variants available from Eppendorf. Please refer to the order information in the Eppendorf Catalog or at www.eppendorf.com.

Your local distributor: www.eppendorf.com/contact
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www.eppendorf.com