DeepChek® Assay

HIV-1 Full PR/RT/INT Drug Resistance

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.

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Contents

Application

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The ***DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance*** is a single tube system which utilizes PCR technology for amplifying the human immunodeficiency virus protease, reverse transcriptase and integrase regions.

This nucleic acid amplification method screens the emergence of mutations in the *Pol* gene of human immunodeficiency virus, type one (HIV-1) specimens. The ***DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance*** can be used to process specimens with viral loads between 100 and 10.000.000 copies per milliliter (cp/mL).

The ***DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance*** 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 HIV-1 Full PR/RT/INT Drug Resistance*** is a reverse transcriptase-polymerase chain reaction test which includes primers, reverse and forward, designed to amplify HIV-1 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 HIV-1 Full PR/RT/INT Drug Resistance*** is performed on a PCR instrument.

Subsequently, the amplicons can be used for sequencing and analyzed with a downstream analysis software to list in a report HIV-1 genome mutations according to available public reference knowledge databases.

Genotypic analysis of this region of HIV-1 facilitates the study of the relationship between mutations and viral resistance to anti-retroviral drugs, specifically the protease (PR), reverse transcriptase (RT) and integrase (INT) inhibitors.

Assay components

The ***DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance*** is provided in one model of 24 reactions (reference: 198A24).

*Table 1 : Volumes and storage conditions of the **198A24 V1***

Label	Volume for 24 tests	Color cap	Storage
RT-PCR			
Master Mix SF	200 µL	1 (green)	-25°C to - 15 °C
Enzyme IV	15 µL	1 (pink)	-25°C to - 15 °C
PR-RT-INT FOR RT-PCR Primers (10µM)	35 µL	1 (yellow)	-25°C to - 15 °C
PR-RT-INT REV RT-PCR Primers (10µM)	35 µL	1 (brown)	-25°C to - 15 °C
H_2O	500 µL	1 (blue)	-25°C to - 15 °C
Nested PCR			
Master Mix PL	380 µL	1 (orange)	-25°C to - 15 °C
PR-RT-INT FOR Nested PCR Primers (10µM)	20 µL	1 (red)	-25°C to - 15 °C
PR-RT-INT REV Nested PCR Primers (10µM)	20 µL	1 (black)	-25°C to - 15 °C
Nested H_2O	500 µL	1 (blue)	-25°C to - 15 °C

Master Mix SF	Enzyme IV		Master Mix PL	
PR-RT-INT FOR RT-PCR Primers	PR-RT-INT REV RT-PCR Primers		PR-RT-INT FOR Nested PCR Primers	PR-RT-INT REV Nested PCR Primers
H_2O			Nested H_2O	

*Figure 1: Disposal of the assay components for the **198A24 V1***

Reagent storage and handling

The ***DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance*** 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
- 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 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 refer to relevant the 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 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.

RNA Extraction

To achieve optimal and sensitive HIV RNA analysis, 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.

The ***DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance*** 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:

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.

PCR reaction setup

1. Incubate the RNA for 5 min at 65 °C and then put the ARN in the ice.
2. Thaw extracted template RNA, RT-PCR primer solutions, RT-PCR Master Mix, and H_2O (RNase-free water) and place them on ice. Load all the tubes into the centrifuge. Spin the specimens at 11.000g for 10 seconds. And then pipette up and down the mix several times before the dispensing.
3. Prepare the “PR-RT-INT” master mix according to the next table. The master mix typically contains all the components required for the RT-PCR except the template RNA. 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 RT-PCR

Reagent	Volume / Reaction
Master Mix SF	6.25 µL
Enzyme IV	0. 25 µL
PR-RT-INT FOR RT-PCR Primers	1.00 µL
PR-RT-INT REV RT-PCR Primers	1.00 µL
H_2O	11.50 µl
TOTAL	**20.00 µL**

4. Vortex the master mix thoroughly and dispense 20 µL into PCR tubes. Mix by pipetting the master mix up and down a few times.
5. Add 5 µL of RNA in the PCR tubes. Mix by pipetting the master mix up and down a few times.
6. Program the thermal cycler according to the program in the next table.

Table 3: RT-PCR cycling program for the PR-RT-INT

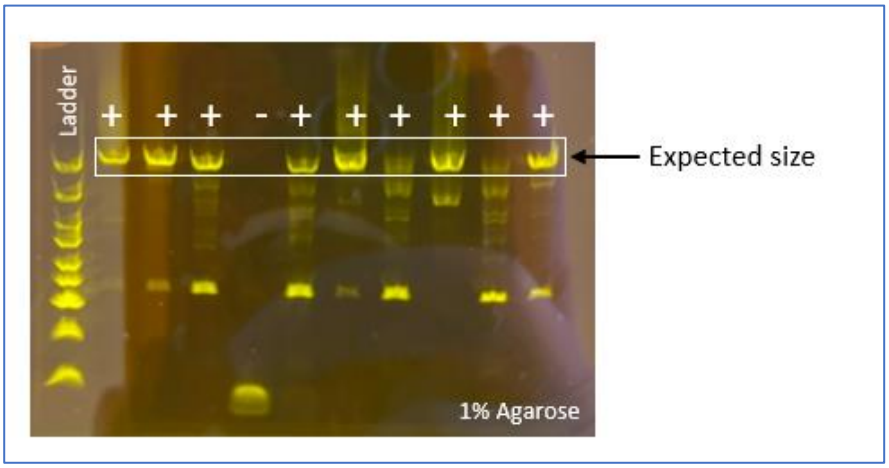
Cycle	Temperature (°C)	Time
RT step	50	10 min
Enzyme activation	98	2 min
10 cycles	98	10 sec
	62 to 52 (delta -1°C/cycle)	10 sec
	72	2 min
30 cycles	98	10 sec
	52	10 sec
	72	2 min
Final extension	72	5 min
	10	∞

Note: the ramping rate shall be 6.0°C/sec.

7. Start the ***DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance*** 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.
8. RT-PCR products shall be controlled through electrophoresis on an 1% 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, please read the troubleshooting section hereinafter.

Note: the expected amplicon size is for the **full PR/RT/INT ≈ 3760 bp**.

Figure 2: example of PR/RT/INT DNA concentrations after the RT-PCR step



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 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 DNA evaporation	Ensure that your (capillary) gel electrophoresis is started immediately after sample preparation. 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 RT-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 HIV-1 test.
		• If > 1'000 cp/mL, do a new RNA isolation on fresh or frozen specimen and start again the whole protocol.
		  ○ If negative again, then run the Nested PCR protocol.
		• If > 100 cp/ML and < 1'000 cp/mL, please improve the conditions of the specimen (e.g., ultra-centrifugation, specimen concentration) and do a new RNA isolation on fresh or frozen specimen and start again the whole protocol.
		  ○ If < 100 cp/mL, then run the Nested PCR protocol.

c.	Limitation variant	If you get repeatedly a negative result, and exclude use errors, it could be a variant binding the reverse transcription binding sites. Contact our technical support.

Note: as if processed in a normal laboratory workflow, a specimen showing two consecutive full negative PCR (no amplification) is then considered as "no result obtained". You shall assess the viral load of the HIV-1 assay and the RNA quality. You can contact ABL Technical Support for further assistance.

Optional Nested PCR reaction setup

1. Thaw the RT-PCR product, Nested PCR primer solutions, Nested Master Mix, Nested H_2O and place them on ice. Load all the tubes into the centrifuge. Spin the specimens at 11.000g for 10 seconds. And then aspirate and discharge the solution several times before the dispensing.
2. Prepare master mix according to the following table. The master mix typically contains all the components required for Nested PCR except the template RT-PCR product. Prepare a volume of master mix greater (n+1) than that required for the total number of reactions to be performed.

Table 4: Reaction components for the Nested PCR

Reagent	Volume / Reaction
Master Mix PL	12.50 µL
PR-RT-INT FOR Nested	0.50 µL
PR-RT-INT REV Nested	0.50 µL
Nested H_2O	9.00 µL
TOTAL	**22.50 µL**

3. Vortex the master mix thoroughly and dispense 22.5 µL into PCR tubes. Mix by pipetting the master mix up and down a few times.
4. Add 2.5 µL of the RT-PCR product 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 the following table.

Table 5: Nested PCR Cycling Program for PR/RT/INT

Cycle	Temperature (°C)	Time
Enzyme activation	94	2 min
10 cycles	98	30 sec
	63 to 53 (delta -1°C/cycle)	10 sec
	72	2 min 30 sec
30 cycles	98	10 sec
	52	10 sec
	72	2 min 30 sec
Final extension	72	5 min
	10	∞

Note: the ramping rate shall be 6.0°C/sec.

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

Note: the expected amplicon size is for the **full PR/RT/INT ≈ 3250 bp**.

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 technics and NGS analyzers vary. NGS sequencing instruments are general laboratory use devices. Sequencing and library preparations reagents are general laboratory use products.

While 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.

Downstream NGS Analysis Software

The sequencing raw data can then be uploaded in a specific downstream software tailored for HIV-1 genome analysis and results. This software can be a standalone medical device and itself can be CE-IVD marked. The software shall rely on recognized and updated public sources about HIV subtyping, HIV tropism and latest knowledge about HIV drug resistance associated mutations.

For the verification studies, NGS files containing nucleotide sequences were analyzed by a downstream analysis software (e.g., the ABL *DeepChek® HIV Software module* (#S-12-023HM) and *license* (#S-12-023HL)). Users shall then follow the software user guide.

Quality controls

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.

Product quality control

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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 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

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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.

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