# *DeepChek® Assay*

# *V3 LOOP / TROPISM*

# V1 (RUO)

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 **103A24 (old reference: (K-17-H2-V3)**

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# Contents

## Application

The ***DeepChek® Assay V3 LOOP / TROPISM*** (RUO) kit is a single tube system which utilizes PCR technology for amplifying the Human Immunodeficiency Virus (HIV-1) V3 loop region from HIV-1 specimens.

This nucleic acid amplification method screens the emergence of HIV-1 genome mutations.

The ***DeepChek® Assay V3 LOOP / TROPISM*** (RUO) is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of PCR, Sanger and next generation sequencing (NGS) workflows.

## Principles of the assay

The ***DeepChek® Assay V3 LOOP / TROPISM*** (RUO) 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 V3 LOOP / TROPISM*** (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 HIV genotypes according to available public reference knowledge databases.

## Assay components

***The DeepChek® Assay V3 LOOP / TROPISM (RUO)*** is provided in one model of 24 reactions (REF 103A24 / OLD REF K-17-H2-V3).

| Label | Volume for 24 Rxn. | Color cap | Storage |
|---|---|---|---|
| **RT-PCR** | | | |
| **Master Mix 2X** | 400 µL | Green | -25°C to - 15 °C |
| **RT Mix** | 12 µL | Orange | -25°C to - 15 °C |
| **TROP FOR RT-PCR Primers** | 45 µL | Yellow | -25°C to - 15 °C |
| **TROP REV RT-PCR Primers** | 45 µL | Yellow | -25°C to - 15 °C |
| **TROP SEQUENCING FOR RT-PCR Primers** | 45 µL | Red | -25°C to - 15 °C |
| **TROP SEQUENCING REV RT-PCR Primers** | 45 µL | Red | -25°C to - 15 °C |
| **Nested PCR** | | | |
| **Master Mix 2X** | 400 µL | Green | -25°C to - 15 °C |
| **TROP FOR Nested PCR Primers** | 45 µL | Yellow | -25°C to - 15 °C |
| **TROP REV Nested PCR Primers** | 45 µL | Yellow | -25°C to - 15 °C |
| **TROP SEQUENCING FOR Nested PCR Primers** | 45 µL | Red | -25°C to - 15 °C |
| **TROP SEQUENCING Nested PCR Primers** | 45 µL | Red | -25°C to - 15 °C |
| **$H_2O$** | 500 µL | Blue | -25°C to - 15 °C |

*Table 1: Volumes and storage conditions of the **DeepChek® Assay V3 LOOP / Tropism (RUO)***

## Reagent storage and handling

The ***DeepChek® Assay V3 LOOP / TROPISM*** **(RUO)** is shipped with dry ice and should 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
- 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.
- Please 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 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 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 V3 LOOP / TROPISM (RUO)*** will work with at least an extraction of 400 µL of specimen (i.e., plasma, serum, whole-blood), to be eluted in 100 µL.

1. For specimens with low viral load, we recommend: To perform an ultracentrifugation procedure. Pellet the specimen 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 Workflow

1. Thaw extracted template RNA, primer solutions, 2x Master Mix and RNase-free water and place them on ice and place them on ice. Load all the tubes into the centrifuge. Spin the samples at 10000 RPM for 10 seconds. And then pipette up and down the mix several times before the dispensing.
2. Prepare V3 loop master mix according to **Table 2**. The master mix typically contains all the components required for 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.

| Reagent | Volume / Reaction |
|---|---|
| Master Mix 2X | 12.50 µL |
| TROP FOR RT-PCR Primers 10 µM | 1.25 µL |
| TROP REV RT-PCR Primers 10 µM | 1.25 µL |
| RT Mix | 0.25 µL |
| **Final Volume** | 15.25 µL |

*Table 2: Reaction components for the **DeepChek® Assay V3 LOOP / Tropism (RUO)** RT-PCR targets*

3. Vortex the master mix thoroughly and dispense 15.25 µL into PCR tubes. Mix by pipetting the master mix up and down a few times.
4. Add 9.75 µL of RNA 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 |
|---|---|---|
| RT step | 50 | 10 min |
| Enzyme activation | 95 | 5 min |
| 45 cycles | 95 | 30 sec |
| | 50 | 30 sec |
| | 68 | 1 min |
| Final extension | 72 | 10 min |
| 1 | 10 | ∞ |

*Table 3: **DeepChek® Assay V3 LOOP / Tropism (RUO)** RT-PCR cycling program*

6. Start the **DeepChek® Assay V3 LOOP / Tropism (RUO) RT-PCR**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.
7. **[Recommended]** - RT-PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of the signal. Even if low-intensity bands usually leads to a successful sequencing, it is recommended to avoid the process if no band can be observed. In that case, first use the **DeepChek® Assay V3 LOOP / Tropism (RUO)** Nested PCR reagents.

**Expected amplicons size for V3 loop region: 585 bp**

## Nested PCR Step-by-Step Workflow for V3 loop (optional)

1. Thaw the RT-PCR product, Nested PCR primer solutions, 2x Master Mix and RNase-free water and place them on ice. Load all the tubes into the centrifuge. Spin the specimens at 11000 g during 10 seconds. And then aspirate and discharge the solution several times before the dispensing.
2. Prepare **DeepChek® Assay V3 LOOP / Tropism (RUO)** master mix according to **Table 4**. The master mix typically contains all the components required for Nested 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.

| Reagent | Volume / Reaction |
|---|---|
| **Master Mix 2X** | 12.5 µL |
| **TROP FOR Nested-PCR Primers** 10 µM | 1.0 µL |
| **TROP REV Nested-PCR Primers** 10 µM | 1.0 µL |
| **Nested-PCR Enzyme** | 1.0 µL |
| **$H_2O$** | 13.0 µL |
| **Final Volume** | 22.0 µL |

*Table 4: Reaction components for the **V3 loop** Nested PCR target*

3. Vortex the master mix thoroughly and dispense 22 µL into PCR tubes. Mix by pipetting the master mix up and down a few times.
4. Add 3 µL of the **DeepChek® Assay V3 LOOP / Tropism (RUO)** 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 **Table 5**.

| Cycle | Temperature (°C) | Time |
|---|---|---|
| Enzyme activation | 95 | 5 min |
| 35 cycles | 95 | 30 sec |
| | 50 | 30 sec |
| | 72 | 1 min |
| Final extension | 72 | 10 min |
| 1 | 10 | ∞ |

*Table 5: **DeepChek® Assay V3 LOOP / Tropism (RUO)**Nested PCR Cycling Program*

6. Start the **DeepChek® Assay V3 LOOP / Tropism (RUO)** 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 leads to a successful sequencing, it is recommended to avoid the process if no band can be observed.

**Expected amplicons size for the Nested V3 Loop: 373 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 fresh RNA extraction.
2. For specimens with low viral load, we recommend to perform 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.
3. Before sequencing, first make sure your PCR products have been purified. In presence of very large PCR bands on the agarose gel, dilute ($1/10^1$ - $1/10^3$) of the PCR product before sequencing.

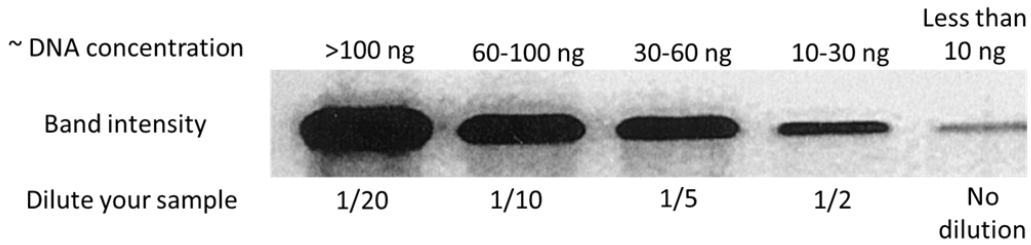


*Figure 1 : DNA concentration examples*

## PCR Products Purification

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

## Sequencing

### Sanger

After the amplicon verification, the specimens are ready for the Sanger sequencing kit processing using the ABL ***DeepDye™ SANGER SEQUENCING REACTION V2*** (24 or 48 reactions) ***(REF 123B24 / 123B48).*** Users shall then follow the user guide.

### NGS

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

Through Illumina:

- **116A24 / 116A48 / 116A96 | ABL DeepChek® NGS LIBRARY PREPARATION V1 (24/48/96 reactions) or**
- **116B24 / 116B48 / 116B96 | ABL DeepChek® NGS LIBRARY PREPARATION V1 (24/48/96 reactions).**
- **124A24 / 124A48 / 124A96|ABL DeepChek® Adapters (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
- **4484355** | Ion 318™ Chip Kit v2

User shall then follow the instructions for use from the manufacturer.

## Data Analysis

### Sanger

AB1 or FASTA files containing nucleotide sequences for V3 Loop/Tropism fragments are analyzed by a downstream analysis software (i.e.the ABL **DeepChek® Software** (#S-12-023) and by the ABL **ViroScore® Software** (#S-09-14)). Users shall then follow the **DeepChek®** and the **ViroScore®** user guides.

### NGS

NGS files containing nucleotide sequences for V3 Loop/Tropism fragments are analyzed by a downstream analysis software (i.e., the ABL **DeepChek® Software** (#S-12-023)). 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 |  | Negative control |
|  | Catalog number |  | Positive control |
|  | Use by |  | Temperature limitation |
|  | Manufacturer |  | Serial Number |
|  | Country and date 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: 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 and distributors

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