altona DIAGNOSTICS

Instructions for use

AltoStar®
HIV RT-PCR Kit 1.5

02/2022 EN

AltoStar®

AltoStar®

HIV RT-PCR Kit 1.5

For use with

CFX96™ Deep Well Dx System (Bio-Rad)

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IVD

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Content

1. About these instructions for use

These instructions for use guide the user in utilizing the AltoStar® HIV RT-PCR Kit 1.5 on the AltoStar® Automation System AM16 (Hamilton; in the following summarized as AltoStar® AM16) with the AltoStar® Connect software (Version 1.7.4 or higher, Hamilton) for automated PCR setup and on the CFX96™ Deep Well Dx System (Bio-Rad, in the following summarized as CFX96™ DW Dx) with the CFX Manager™ Dx software (Version 3.1, Bio-Rad) for real-time PCR. For details on the use of the AltoStar® AM16, the AltoStar® Connect software, the AltoStar® Purification Kit 1.5, the AltoStar® Internal Control 1.5 and the CFX96™ DW Dx, refer to the respective instructions for use listed below:

- AltoStar® Automation System AM16 Operator's Manual IVD (Hamilton)
- AltoStar® Connect Software Manual IVD (Hamilton)
- Instructions for use AltoStar® Purification Kit 1.5
- Instructions for use AltoStar® Internal Control 1.5
- CFX96™ Dx and CFX96™ Deep Well Dx Systems Operation Manual (Bio-Rad)

Throughout this manual, the terms CAUTION and NOTE have the following meanings:

CAUTION

Highlights operating instructions or procedures which, if not followed correctly, may result in personal injury or impact product performance. Contact altona Diagnostics technical support for assistance.

NOTE

Information is given to the user that is useful but not essential to the task at hand.

Read the instructions for use carefully before using the product.

2. Intended use

The AltoStar® HIV RT-PCR Kit 1.5 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of human immunodeficiency virus type 1 (HIV-1) specific RNA in human EDTA plasma. It is configured for use with the CFX96™ Deep Well Dx System (Bio-Rad) in combination with the AltoStar® Automation System AM16, the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5.

The AltoStar® HIV RT-PCR Kit 1.5 is intended to be used for viral load monitoring in individuals with HIV infection.

The results generated with the AltoStar® HIV RT-PCR Kit 1.5 have to be interpreted in conjunction with other clinical and laboratory findings.

The AltoStar® HIV RT-PCR Kit 1.5 is intended for use by professional users trained in molecular biological techniques and *in vitro* diagnostic procedures.

3. Kit content

The AltoStar® HIV RT-PCR Kit 1.5 contains the following components:

Table 1: Kit components

Lid color	Component	Number of tubes	Nominal volume [µl/tube]
Blue	Master A[1)]	8	240[2)]
Purple	Master B[1)]	8	300[3)]
Red	QS1[4)]	2	550
Red	QS2[4)]	2	550
Red	QS3[4)]	2	550
Red	QS4[4)]	2	550
White	NTC[5)]	2	550

[1)] Contains biological material of animal origin

[2)] Contains an additional volume of 40 µl to compensate the dead volume of the AltoStar® AM16's liquid handling

[3)] Contains an additional volume of 52 µl to compensate the dead volume of the AltoStar® AM16's liquid handling

[4)] Quantification Standard (Positive Control)

[5)] No Template Control (Negative Control)

CAUTION

Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, product performance could be compromised.

The AltoStar® HIV RT-PCR Kit 1.5 contains enough reagents to perform 96 reactions in a maximum number of 8 runs.

The product is shipped on dry ice. Upon receipt and before first use, check the product and its components for:

- Integrity
- Completeness with respect to number, type and filling
- Correct labeling
- Expiration date
- Frozen state
- Clarity and absence of particles

If one or more product components are not frozen upon receipt, if tubes have been compromised during shipment or are missing, contact altona Diagnostics technical support for assistance (see chapter 11. Technical support).

4. Storage and handling

All reagents included in the AltoStar® HIV RT-PCR Kit 1.5 are ready-to-use solutions.

4.1 Storage

All components of the AltoStar® HIV RT-PCR Kit 1.5 must be stored at -25 °C to -15 °C upon arrival.

CAUTION

Improper storage conditions could compromise product performance.

CAUTION

Do not use products beyond the expiration date. The use of expired products could compromise product performance.

4.2 Handling

CAUTION

Do not exceed thaw-freeze-sequence and handling durations specified in these instructions for use, as this could compromise product performance.

CAUTION

Improper handling of product components and samples may cause contamination and could compromise product performance:

- Do not interchange vial or bottle caps.
- Store positive and/or potentially positive material separated from the kit components.
- Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
- Always dispose gloves after handling positive and/or potentially positive material.
- Do not open the PCR plates post amplification.

CAUTION

Do not mix components from different kit lots. The use of different kit lots could compromise product performance.

4.2.1 Master A and Master B

After thawing, Master A and Master B are stable for 5 hours at up to +30 °C.

NOTE

If Master A and Master B were thawed but not used, they can be refrozen and thawed again once for later runs. If opened, discard the lids and use new lids to avoid contamination of the reagents.

4.2.2 Quantification Standards and No Template Control

1. After thawing, the Quantification Standards (QSs) and the No Template Control (NTC) are stable for 5 hours at up to +30 °C.
2. Discard the lids of the QSs and NTC tubes at each use and use new lids to avoid contamination of the reagents.
3. After use close the QSs and NTC tubes with new lids and freeze them immediately.
4. Do not exceed the following thaw-freeze-sequence for each QS and NTC tube: *Thaw 1 → Freeze 1 → Thaw 2 → Freeze 2 → Thaw 3 → Freeze 3 → Thaw 4*

5. Product description

The AltoStar® HIV RT-PCR Kit 1.5 is an *in vitro* diagnostic test for the detection and quantification of HIV-1 specific RNA (groups M, N and O) in human EDTA plasma.

It is based on real-time RT-PCR technology, utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of HIV specific target sequences and fluorescently labeled target specific probes for the detection of the amplified DNA.

In addition to the HIV RNA specific amplification and detection system the assay includes oligonucleotides for the amplification and detection of the internal control (in the following abbreviated as IC; AltoStar® Internal Control 1.5).

Probes specific for HIV RNA are labeled with the fluorophore FAM™. The probe specific for the IC is labeled with a fluorophore (JOE™) detectable in the VIC™ channel.

Using probes linked to distinguishable dyes enables the parallel detection of HIV specific RNA and the IC in corresponding detection channels of the CFX96™ DW Dx.

5.1 Background

The human immunodeficiency virus (HIV) belongs to the genus Lentivirus, which is a subgroup within the family of *Retroviridae*. There are two described species of HIV, i.e., HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 is the most common strain comprising a positive-sense single-stranded RNA genome which is approximately 9.8 kb in length [1]. HIV-1 can be subdivided into a major (namely group M) and at least two minor groups (namely group N and O). A high genetic variability of the genome is the fundamental driver in the evolution of HIV [2], although the viral genome encodes a low number of genes which necessitates the exploitation of host-cell proteins by the virus. HIV gains entry to CD4 expressing (CD4+) immune cells such as helper T cells, macrophages, and dendritic cells [3]. In infected CD4+ host cells, the viral RNA genome is converted into DNA via reverse transcription prior to the viral assembly.

Epidemiologically, HIV has caused 1,7 million (1,4 million – 2,3 million) new infections (all ages) globally in 2018. Although HIV infections are diagnosed worldwide, the highest prevalence is located in sub-Saharan Africa [4]. The transmission of HIV occurs primarily sexually (contact with blood, pre-ejaculate, semen and vaginal fluids), however, HIV infections are also caused by contaminated blood transfusion, the reuse of injection needles, perinatal transmission at pregnancy, and breastfeeding [5].

Patients suffering from an HIV infection may experience a short acute phase with flu-related symptoms. Non-treated infections lead to an acquired immunodeficiency syndrome (AIDS), which is characterized by the progressive failure of the immune system [6]. The depletion of CD4+ immune cells enables the spreading of opportunistic infections and tumors in immunocompromised patients [3]. Typical conditions of AIDS are respiratory tract infections, pneumocystis pneumonia and cachexia.

Since there is no cure for HIV infections, the medical treatment of HIV relies on the containment of the infection by an antiretroviral therapy (ART) [5]. The monitoring and reduction of the viral load leads to a chronic condition preventing the progression towards AIDS [7]. To date, there is no vaccination available.

5.2 Component description

5.2.1 Master A and Master B

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, as well as the PCR mediated amplification and target detection of HIV specific RNA and of the IC in one reaction setup.

5.2.2 Quantification Standards

The QSs contain standardized concentrations of HIV specific RNA (see table 2). They were calibrated against the 4th WHO International Standard for HIV-1 RNA (NIBSC code: 16/194; subtype B). The QSs are used to verify the functionality of the HIV RNA specific amplification and detection system as well as to generate a standard curve, which allows the quantification of HIV specific RNA in a sample.

Table 2: Quantification Standards

Quantification Standard	Concentration [IU/µl]
QS1	1.00E+04
QS2	1.00E+03
QS3	1.00E+02
QS4	1.00E+01

NOTE

The unit of the concentration of the QSs is international units (IU). Conversion to copies is possible by using the following conversion factor: 0.42 copies/IU (1 IU = 0.42 copies).

5.2.3 No Template Control

The NTC contains no HIV specific RNA but does contain the IC template. The NTC is used as negative control for the HIV RNA specific real-time RT-PCR and indicates possible contamination of Master A and Master B.

5.3 AltoStar® Workflow

The AltoStar® Workflow comprises the following IVD products:

- AltoStar® Automation System AM16 (Hamilton)
- AltoStar® Connect software version 1.7.4 or higher (Hamilton)
- CFX96™ Deep Well Dx System (Bio-Rad) with CFX Manager™ Dx software version 3.1 (Bio-Rad)

The workflow includes the following steps:

1. Programming an AltoStar® run.
2. Purification run on the AltoStar® AM16 using the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5.
3. RT-PCR setup run on the AltoStar® AM16 using the AltoStar® HIV RT-PCR Kit 1.5.
4. Real-time RT-PCR run on a CFX96™ DW Dx.

For further details on steps 3 and 4 of the workflow, refer to chapter 7. Using the AltoStar® HIV RT-PCR Kit 1.5 with the AltoStar® Workflow. All sample types and sample volumes specified for use with the AltoStar® Purification Kit 1.5 can be processed simultaneously on the AltoStar® AM16. Each sample can be analyzed with as many real-time PCR assays in parallel as the available eluate allows.

NOTE

Assays with differing PCR temperature profiles are automatically sorted to separate PCR plates.

5.4 Samples

5.4.1 Sample types

The following sample type is validated for use with the AltoStar® HIV RT-PCR Kit 1.5:

- Human EDTA plasma

CAUTION

Do not use other sample types! The use of other sample types could compromise product performance.

5.4.2 Sample collection and handling

Blood has to be collected with commercially available standard blood collection systems (e.g. Sarstedt, Becton Dickinson, Greiner or equivalent). Tube contents should be mixed directly after sample collection. The blood samples should be shipped cooled at +2 °C to +8 °C. Transport should occur following the local and national instructions for the transport of biological material.

For generation of EDTA plasma, whole blood should be centrifuged according to the instructions provided by the manufacturer of the collection system within 24 hours after collection. Before use EDTA plasma should not be stored for more than 48 hours at room temperature (+20 °C to +25 °C), 5 days at +2 °C to +8 °C or 2 months at -25 °C to -15 °C.

CAUTION

Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

NOTE

Frozen storage of samples does not compromise kit performance. When working with frozen samples, make sure samples are completely thawed and properly mixed before use.

6. Warnings, precautions and limitations

- Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, product performance could be compromised.
- Improper storage conditions could compromise product performance.
- Do not use products beyond the expiration date. The use of expired products could compromise product performance.
- Do not exceed thaw-freeze-sequence and handling durations specified in these instructions for use, as this could compromise product performance.
- Improper handling of product components and samples may cause contamination and could compromise product performance:
  - Do not interchange vial or bottle caps.
  - Store positive and/or potentially positive material separated from the kit components.
  - Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
  - Always dispose gloves after handling positive and/or potentially positive material.
  - Do not open the PCR plates post amplification.
- Do not mix components from different kit lots. The use of different kit lots could compromise product performance.
- Do not use other sample types! The use of other sample types could compromise product performance.
- Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

- Storage of eluates under wrong conditions may lead to degradation of the HIV target sequence and could compromise product performance.
- Do not use an assay protocol version other than the one indicated on the 2D barcode in these instructions for use. Using an incorrect assay protocol version could compromise product performance.
- A lack of centrifugation of the product components after thawing may cause contamination with reagent residues in the lids and could compromise product performance.
- Do not reuse tube caps to avoid contamination of the reagents, as this could compromise product performance.
- As with any diagnostic test, results shall be interpreted in consideration of all clinical and laboratory findings.
- The presence of PCR inhibitors (e.g. heparin) could cause false negative or invalid results.
- Do not exceed the PCR mix storage time, as this could compromise product performance.
- In case the sample contains other pathogens than HIV, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.
- Disposal of hazardous and biological waste shall comply with local and national regulations to avoid environmental contamination.
- Potential mutations within the target regions of the HIV genome covered by primers and/or probes used in the kit may result in underquantification and/or failure to detect the presence of the pathogen.

7. Using the AltoStar® HIV RT-PCR Kit 1.5 with the AltoStar® Workflow

The following part of these instructions for use describes the use of the AltoStar® HIV RT-PCR Kit 1.5 in conjunction with the AltoStar® Workflow. The AltoStar® Workflow comprises different IVD products (AltoStar® AM16, the AltoStar® Connect software, the AltoStar® Purification Kit 1.5, the AltoStar® Internal Control 1.5 and the CFX96™ DW Dx). Use of those products is described in detail in the respective instructions for use.

- AltoStar® Automation System AM16 Operator's Manual IVD (Hamilton)
- AltoStar® Connect Software Manual IVD (Hamilton)
- Instructions for use AltoStar® Purification Kit 1.5
- Instructions for use AltoStar® Internal Control 1.5
- CFX96™ Dx and CFX96™ Deep Well Dx Systems Operation Manual (Bio-Rad)

7.1 Sample volume

The AltoStar® HIV RT-PCR Kit 1.5 is validated for nucleic acid purifications from a sample volume of 1,000 µl using the AltoStar® AM16. Additional sample volume has to be provided to account for the dead volume of the sample tube used (see chapter 7.2 Sample tubes).

NOTE

Up to 48 samples of 1,000 µl can be processed simultaneously in one purification run.

7.2 Sample tubes

Sample tubes suitable for use on the AltoStar® AM16 can be purchased from altona Diagnostics (7 ml tube with cap, 82 x 13 mm, VK000010). Other sample tubes can be tested for applicability by the user. For details, refer to the instructions for use of the AltoStar® Purification Kit 1.5.

7.3 Sample barcodes

For automated sample identification by the AltoStar® AM16 all sample tubes must be labeled with a suitable barcode. For details, refer to the instructions for use of the AltoStar® Purification Kit 1.5.

7.4 Material and devices required but not provided

The material and devices shown in table 3 must be ordered from altona Diagnostics.

Table 3: Required material and devices

Material	Description	Order No.
AltoStar® Molecular Diagnostic Workflow	Product bundle containing the AltoStar® Automation System AM16, the AltoStar® Connect software (Version 1.7.4 or higher) and IT hardware	AM16
AltoStar® Detection	Product bundle containing the CFX96™ Deep Well Dx System with CFX Manager™ Dx Software (Version 3.1), a barcode scanner and IT hardware	DT16
AltoStar® Purification Kit 1.5	Nucleic acid isolation and purification chemistry for use with the AltoStar® Automation System AM16	PK15-16/ PK15-46
AltoStar® Internal Control 1.5	Nucleic acid extraction and PCR amplification and detection control	IC15-16/ IC15-46
PCR Plate	Semi-skirted, barcoded 96 multi-well plate with white wells	VK000005
PCR Plate Sealing Foil	Sealing foil for the PCR plate	VK000006
1,000 µl CO-RE Tips	1,000 µl filter tips for use with the AltoStar® Automation System AM16	VK000007
300 µl CO-RE Tips	300 µl filter tips for use with the AltoStar® Automation System AM16	VK000008
Pooling Tube	Barcoded tube for pooling of PCR reagents	VK000002

Material	Description	Order No.
Waste Bag	Autoclavable sterile bag for use with the AltoStar® Automation System AM16	VK000009
Screw Cap - red	Screw cap for QS1–QS4 tubes	VK000012
Screw Cap - blue	Screw cap for Master A tubes	VK000013
Screw Cap - purple	Screw cap for Master B tubes	VK000015
Screw Cap - white	Screw cap for NTC tubes	VK000016

Table 4: Additional laboratory material and devices

Material	Description	Order No.
Plate Sealer	e.g. AltoStar® Plate Sealer	VK000023
	e.g. PX1 Plate Sealer (Bio-Rad)	VK000033

7.5 General material and devices

- Vortex mixer
- Powder-free gloves (disposable)
- Centrifuge for centrifugation of the AltoStar® HIV RT-PCR Kit 1.5 components
- Centrifuge for centrifugation of PCR plates

7.6 Procedure

7.6.1 Overview of the AltoStar® Workflow

The steps of the complete AltoStar® Workflow are summarized in table 5. Information on specific settings for use with the AltoStar® HIV RT-PCR Kit 1.5 are provided in chapter 7.6.2 Programming an AltoStar® run. For detailed instructions for steps 1–5, refer to the instructions for use of the AltoStar® Purification Kit 1.5, the AltoStar® Connect software and the AltoStar® AM16.

Steps 6–11 are described in more detail in chapters 7.6.3 Starting a PCR setup run to 7.6.10 Export of PCR results for manual result interpretation.

Table 5: Overview of the AltoStar® Workflow

Step	Action
1. Start the AltoStar® AM16	• Switch on the AltoStar® AM16. • Switch on the computer and the monitor. • Start the AltoStar® Connect software.
2. Perform maintenance	• In the menu bar click **Application** → **Instrument Maintenance**. ◦ If weekly maintenance is due, click **Start Weekly Maintenance**. ◦ If daily maintenance is due, click **Start Daily Maintenance**. • Follow the on-screen instructions for the maintenance process.
3. Program an AltoStar® run	• In the menu bar click **Program Run** → **Program Run** (**AltoStar® Purification**). Alternatively, go back to the Start screen and click the **Program Run** button. • Enter samples or import from the LIMS. • Select the following assay for the samples unless already imported from the LIMS: ◦ AltoStar® HIV RT-PCR Kit 1.5 • Click the **Create Run** button in the tool bar to create the AltoStar® run.

Step	Action
4. Start a purification run	• In the menu bar click **Purification** → **Start Purification**. Alternatively, go back to the Start screen and click the **Start Purification** button. • Select the purification run to be started to display the samples included in the selected purification run. • Prepare the purification reagents: ◦ Ensure that the purification reagents to be used have the same loading number (except AltoStar® Internal Control 1.5) and are not expired. ◦ If precipitates are visible in the Lysis Buffer, heat it (≤ +50 °C) until completely dissolved. ◦ Thaw the IC (AltoStar® Internal Control 1.5) and vortex for 5 seconds. ◦ Vortex the Magnetic Beads for 5 seconds without wetting the lid. • Prepare the samples for the purification run to be started as described in the instructions for use of the AltoStar® Purification Kit 1.5. • Click the **Start Run** button in the tool bar. • Follow the loading dialogs and load the instrument accordingly. • Confirm the Loading complete message with **OK** or wait 10 seconds. The system will now perform the purification run automatically.
5. Finish the purification run	• Make sure the loading tray is empty and confirm the Run finished dialog with **OK**. • Follow the instructions in the Maintenance dialog and confirm with **OK**. • Seal and store the components of the AltoStar® Purification Kit 1.5 that can be reused. The eluates in the unsealed eluate plate are stable at room temperature (max. +30 °C) for a total of 4 hours. • If the associated PCR setup run is not started right away, seal the eluate plate with an Eluate Plate Sealing Foil and store at +2 °C to +8 °C for up to 24 hours. • View the purification run results to confirm successful processing of each sample.

Step	Action
6. Start a PCR setup run	• In the menu bar, click **PCR Setup → Start PCR Setup**. Alternatively, go back to the Start screen and click the **Start PCR Setup** button. • Select the PCR setup run to be started in order to display the eluate plate and reagents included in the selected PCR setup run. • Prepare the PCR reagents: ◦ Ensure that masters and controls to be used are from the same kit lot and are not expired. ◦ Thaw the required amount of master and control tubes, vortex briefly and spin down in a centrifuge. • If the eluate plate is sealed, briefly centrifuge the plate and unseal carefully. • Click the **Start Run** button in the tool bar. • Follow the Loading dialog and load the instrument accordingly. • Confirm the Loading complete message with **OK** or wait 10 seconds. The system will now perform the PCR setup run automatically.
7. Finish the PCR setup run	• Make sure the loading tray is empty and confirm the Run finished dialog with **OK**. • Follow the instructions in the Maintenance dialog and confirm with **OK**. • Close and store the components of the AltoStar® HIV RT-PCR Kit 1.5 that can be reused. • View the PCR setup run results to confirm successful processing of each sample.
8. Seal the PCR plate	• Seal the PCR plate with a PCR Plate Sealing Foil.

Step	Action
9. Start the PCR run	• Switch on the CFX96™ DW Dx, the attached computer and the monitor. • Start the CFX Manager™ Dx software. • Open the CFX96™ DW Dx. • Centrifuge the PCR plate and insert it into the CFX96™ DW Dx. • Select **File** → **Open** → **LIMS File…** from the menu bar. • Scan the barcode of the PCR plate with the handheld barcode scanner. • Close the CFX96™ DW Dx. • Click the **Start Run** button to start the PCR run. Name and save the PCR run file. The CFX96™ DW Dx will now perform the PCR run automatically.
10. Separate assays for individual analysis	• Separate all assays in the PCR run into distinct well groups.
11. Analyze the data and interpret the PCR run results	For each well group individually: • Perform baseline correction in all wells for all detection channels used. • Exclude wells with irregular PCR signals. • Set the thresholds of all detection channels according to the controls. • Exclude wells containing invalid data. • Generate the LIMS result file for export of results to the LIMS. • Generate the result report for manual result interpretation.

CAUTION

Storage of eluates under wrong conditions may lead to degradation of the HIV target sequence and could compromise product performance.

7.6.2 Programming an AltoStar® run

For detailed information on starting an AltoStar® run, refer to the instructions for use of the AltoStar® Purification Kit 1.5, the AltoStar® Connect software and the AltoStar® AM16. Specific settings for use with the AltoStar® HIV RT-PCR Kit 1.5 are listed below:

- For quantitative assay application QS1–4 and NTC are selected and for qualitative assay application QS4 and NTC are selected.
- The required sample volume is 1,000 µl plus the dead volume for the respective sample tube (see chapters 7.1 Sample volume and 7.2 Sample tubes).
- The required eluate volume for the AltoStar® HIV RT-PCR Kit 1.5 is 45 µl.
- Make sure that the correct assay protocol version is used for the run. For information on the current protocol version see chapter 15. Assay protocol for the AltoStar® Connect software and information for LIMS integration. The respective assay protocol is encoded in the 2D barcode displayed there. For information on purification and assay protocol import into the AltoStar® Connect software, refer to the respective instructions for use.

CAUTION

Do not use an assay protocol version other than the one indicated on the 2D barcode in these instructions for use. Using an incorrect assay protocol version could compromise product performance.

7.6.3 Starting a PCR setup run

1. Select **PCR Setup** → **Start PCR Setup** in the menu bar. Alternatively, go back to the Start screen of the AltoStar® Connect software and select the **Start PCR Setup** button. The Start PCR Setup Run screen is displayed.

The pending PCR setup runs are displayed in the Programmed PCR Setup Runs table on the left side of the screen.

2. Select the PCR setup run to be started in the Programmed PCR Setup Runs table.

   - The samples included in the selected PCR setup run are displayed in the table on the top right side of the screen (Samples in selected PCR Setup Run).
   - The QSs and controls required for the selected PCR setup run are displayed in the table on the middle right side of the screen (Controls in selected PCR Setup Run).
   - The number of master tubes required for the selected PCR setup run is displayed in the table on the bottom right side of the screen (Required master tubes for the selected PCR Setup Run).

NOTE

The number of prioritized samples in a PCR setup run is displayed in the column **No. of prioritized Samples**. Conduct PCR setup runs with prioritized samples first to facilitate fastest processing of prioritized samples.

Before clicking the **Start Run** button in the tool bar, prepare the required reagents as described in chapter 7.6.3.1 Preparing reagents for a PCR setup run. If the eluate plate required for the selected PCR setup run has been sealed for storage prepare it as described in the instructions for use of the AltoStar® Purification Kit 1.5.

7.6.3.1 Preparing reagents for a PCR setup run

1. Thaw the required QSs, controls and the required number of master tubes completely at room temperature (max. +30 °C).
2. Mix the reagents by gentle vortexing.
3. Centrifuge the tubes briefly to remove drops from the lid.

CAUTION

A lack of centrifugation of the product components after thawing may cause contamination with reagent residues in the lids and could compromise product performance.

7.6.3.2 Loading the AltoStar® AM16 for a PCR setup run

For detailed information regarding the loading process, refer to the instructions for use of the AltoStar® Automation System AM16 and the AltoStar® Connect software.

1. Click the **Start Run** button in the tool bar of the Start PCR Setup Run screen to display the Loading dialog.

The Loading dialog consists of a visual representation of the AltoStar® AM16 deck at the top and a table specifying the carriers, the respective tracks on the AltoStar® AM16 deck for each carrier, the material to be loaded onto each carrier and comments with respect to the carrier loading.

NOTE

To visualize the position of an item on a carrier and the position of the carrier on the AltoStar® AM16 deck, select the respective row of the table in the Loading dialog.

The position of the item and its carrier is visualized:

- Highlighted in red in the visual representation of the instrument deck
- On the AltoStar® AM16 by flashing loading lights above the tracks where the selected carrier must be placed

2. Load the required material, the prepared eluate plate and the prepared reagents onto the suitable carriers.

   - Exchange only **completely empty** 1,000 µl tip racks for **completely full** 1,000 µl tip racks on the tip carrier.
   - Exchange only **completely empty** 300 µl tip racks for **completely full** 300 µl tip racks on the tip and plate carrier.

NOTE

Exchange of tip racks, which are not completely empty as well as handling of individual tips may interfere with the automatic tip management and cause run aborts.

   - Place the required eluate plate with well A1 to the left of the black plate position.

- Place a PCR plate with well A1 to the left of the silver front plate position.
- Load a tube carrier 24 with one unused pooling tube for each assay in the PCR setup run.
- Gently push the tubes all the way to the bottom of the carrier and rotate the tubes until the tube barcodes are visible through the carrier window.
- Load the reagent tube carrier 32 with the assay components required for the PCR setup run.
- Gently push the tubes all the way to the bottom of the carrier and rotate the tubes until the tube barcodes are visible through the carrier window.

NOTE

The position of the individual tubes on the carriers is arbitrary.

NOTE

The volume of the loaded components is not checked by the system prior to processing. Insufficient component volume will prevent successful PCR setup for the affected assay.

NOTE

Starting a PCR setup run with lids still on the tubes may cause the run to abort during processing.

3. Load the carriers with the carrier barcode towards the rear facing right.
4. Insert populated carriers into the respective tracks between the front and rear slide blocks of the loading tray until they touch the stop hooks on the far side of the loading tray.

NOTE

Pushing the carriers past the stop hooks may damage the instrument and interfere with the loading process.

5. Check that the tip eject sheet and the tip waste container are in the correct position and a new waste bag is placed in the container.

6. Click **OK** in the Loading dialog to proceed with the loading process.

NOTE

By clicking **Cancel** the PCR setup run will be canceled, but it can be started again (see chapter 7.6.3 Starting a PCR setup run).

The AltoStar® AM16 draws the carriers into the instrument and performs barcode verification.

NOTE

The AltoStar® AM16 automatically verifies:

- Correct type and localization of the loaded carriers
- Correct identity and position of the items loaded on the carriers
- Lot congruence of the components of the individual AltoStar® assay kits
- Non-expiry of all loaded AltoStar® assay components
- Correct positioning of the tip eject sheet

If any of these checks fail, the user is prompted with a message dialog specifying the problem at hand and instructions to correct the issue accordingly. For further information regarding error handling, refer to the instructions for use of the AltoStar® Connect software.

NOTE

Altering positions of any loaded item after the carrier has been drawn into the instrument may result in the abort of the PCR setup run and/or damage to the instrument.

When all checks have passed the Loading complete dialog is displayed.

7. Confirm the Loading complete dialog by clicking **OK** or wait 10 seconds for the automatic start of the process.

NOTE

By clicking **Cancel** the PCR setup run will be canceled, but it can be started again (see chapter 7.6.3 Starting a PCR setup run).

The PCR setup run is started and will be conducted without user intervention.

7.6.3.3 During the PCR setup run

No further user interaction is required until the PCR setup run has finished. The Processing Status screen is displayed showing the status of the PCR setup run and the estimated time remaining.

NOTE

Pushing or pulling carriers or the door of the AltoStar® AM16 during a PCR setup run may abort the run.

7.6.4 End of the PCR setup run

At the end of the PCR setup run the Run finished dialog is displayed.

1. Make sure that the loading tray is empty.
2. Confirm the Run finished dialog by clicking **OK**.

The AltoStar® AM16 will unload the carriers. Make sure not to stand in the way of the unloading carriers.

After unloading the Maintenance dialog is displayed.

3. Follow the instructions of the Maintenance dialog.

The table of the dialog displays the number of reactions in the master tubes that were not used in the PCR setup run.

4. If another PCR setup run using the currently loaded eluate plate is to be started right away, the eluate plate can remain unsealed on the carrier position. If this is **not** the case, seal and store the eluate plate. For further information, refer to the instructions for use of the AltoStar® Purification Kit 1.5.

NOTE

The eluates in the eluate plate are stable at room temperature (max. +30 °C) for a total of 4 hours after completion of the purification run.

5. Close reagent tubes with suitable unused tube caps.

CAUTION

Do not reuse tube caps to avoid contamination of the reagents, as this could compromise product performance.

6. Store reagents for reuse as described in chapter 4.2 Handling.
7. Dispose of the used materials (see chapter 9. Disposal).
8. Confirm the Maintenance dialog by clicking **OK**.

7.6.4.1 PCR setup run results

The PCR setup run results are saved in the AltoStar® Connect software.

1. Click **PCR Setup** → **PCR Setup Results** in the menu bar to access the Results screen.

The Results screen displays a table with all samples used in the latest PCR setup run and a column **Status** at the right showing if the PCR setup process for a given sample was conducted completely (see table 6).

Table 6: PCR setup run results

Status	PCR setup run result
Processed	• The eluate was successfully processed in the PCR setup run. • The resulting RT-PCR mix is ready for use in a PCR run.
Error	• The eluate was not processed successfully. • The respective RT-PCR mix will be automatically omitted in the following PCR analysis.

2. To view the results of prior PCR setup runs, click the **Load** button in the menu bar, select the desired PCR setup run from the list in the opening Load Results dialog and click **OK**.

3 PCR setup run result files are automatically generated by the AltoStar® Connect software:

- A LIMS file (.xml) to pass detailed information about the PCR setup run including results back to the LIMS
- A report (.pdf) containing detailed information about the PCR setup run including results for documentation purposes
- A cycler file (.plrn) for automatic programming of the CFX96™ DW Dx

These files are saved to the location specified in the system settings of the AltoStar® Connect software.

NOTE

PCR setup run result files can be generated again by loading the respective PCR setup run and clicking the **Create LIMS File** button to generate the LIMS file, the **Create Report** button to generate the report or the **Create Bio-Rad Cycler File** button to generate the cycler file.

7.6.5 Sealing of the PCR plate

After completion of the PCR setup run, the PCR plate must be sealed with a PCR Plate Sealing Foil. It is recommended to use the AltoStar® Plate Sealer [4s3™ Semi-Automatic Sheet Heat Sealer (4titude)] or the PX1 PCR Plate Sealer (Bio-Rad). The suitability of plate sealers other than the recommended plate sealers has to be evaluated by the user.

If one of the recommended plate sealers is used for sealing, proceed as follows:

1. Switch on the plate sealer and make sure that the plate adapter is not in the drawer.
2. Ensure that the settings of the plate sealer are as follows:

Table 7: Settings of the plate sealer

Plate sealer	Settings	
	Temperature [°C]	Time [s]
AltoStar® Plate Sealer [4s3™ Semi-Automatic Sheet Heat Sealer (4titude)]	170	2
PX1 PCR Plate Sealer (Bio-Rad)	175	3

3. Wait until the set temperature is reached. This may take several minutes.
4. Place the PCR plate on the plate adapter of the plate sealer.
5. Place one PCR Plate Sealing Foil on the PCR plate so that the print ‘THIS SIDE UP’ is readable. Make sure that all wells of the PCR plate are covered with foil and no well is obscured by the writing.

NOTE

Operating the plate sealer without the plate adapter placed in the drawer may render the sealer nonfunctional. In this case, contact altona Diagnostics technical support for assistance (see chapter 11. Technical support).

NOTE

If the PCR Plate Sealing Foil or the frame is placed incorrectly, the foil may stick to the heating plate within the plate sealer during sealing. This will render the sealer nonfunctional. In this case, or if the sealing step has been initiated without a PCR Plate Sealing Foil, let the plate sealer cool down to room temperature and contact altona Diagnostics technical support for assistance (see chapter 11. Technical support).

6. Assemble the sealing frame on top to hold down the sealing foil.
7. Open the drawer by pressing the **Operate***/ ⏏** button.
8. Place the assembly consisting of the plate adapter, the PCR plate, the PCR Plate Sealing Foil and the sealing frame into the plate sealer and press the **Operate***/ ⏏** button.
9. The drawer closes automatically, seals for the set time and reopens automatically.
10. Take the sealed PCR plate and the plate adapter out of the plate sealer and close the plate sealer by pressing the **Close***/ ⏏** button.

* AltoStar® Plate Sealer [4s3™ Semi-Automatic Sheet Heat Sealer (4titude)]

**PX1 PCR Plate Sealer (Bio-Rad)

7.6.5.1 PCR mix stability

After completion of the PCR setup run the RT-PCR mix in the sealed PCR plate is stable at room temperature (max. +30 °C) for 30 minutes.

CAUTION

Do not exceed the PCR mix storage time, as this could compromise product performance.

7.6.6 Starting a PCR run

The PCR run is performed on a CFX96™ DW Dx under control of the CFX Manager™ Dx software.

1. Switch on the CFX96™ DW Dx, the attached computer and the monitor.
2. Start the CFX Manager™ Dx software.
3. In the menu bar of the CFX Manager™ Dx software select **File** → **Open** → **LIMS File…** to open the Open LIMS File dialog.
4. In the opening Open LIMS File dialog, make sure that the cursor is blinking in the **File name** field at the bottom. If it is not, click into the **File name** field.
5. Scan the PCR plate barcode with the handheld barcode scanner to automatically select and open the correct LIMS file. The Run Setup dialog is displayed.

NOTE

All parameters required for the start of the PCR run are transferred automatically from the AltoStar® Connect software to the CFX96™ DW Dx by means of the cycler file.

6. Click the **Open Lid** button in the Run Setup dialog to open the lid of the CFX96™ DW Dx.
7. Briefly centrifuge the sealed PCR plate to ensure all liquid is at the bottom of the wells.
8. Insert the sealed PCR plate into the heating block of the CFX96™ DW Dx with well A1 to the left side.
9. Close the CFX96™ DW Dx by clicking the **Close Lid** button in the Run Setup dialog.
10. Start the PCR run by clicking the **Start Run** button in the Run Setup dialog.

7.6.6.1 During the PCR run

No user interaction is required until the PCR run has finished. The Run Details dialog is displayed showing the status of the PCR run and the estimated time remaining.

NOTE

Opening the lid of the CFX96™ DW Dx during a PCR run by operating the button at the front of the lid or by clicking **Open Lid** in the Run Details dialog will abort the run and void all results.

At the end of the PCR run the Data Analysis window is displayed, which shows the amplification curves, the plate layout and the results.

7.6.6.2 Assigning assays to well groups

The AltoStar® Workflow processes one or several PCR assays simultaneously on one PCR plate. However, each assay must be analyzed separately by the user according to the instructions for use of the respective assay.

To this end, all assays on a PCR plate must be assigned to individual well groups in the CFX Manager™ Dx software by the user.

1. In the Data Analysis window click the **Plate Setup** button in the tool bar and select **View/Edit Plate**. The Plate Editor dialog is displayed (see figure 1).

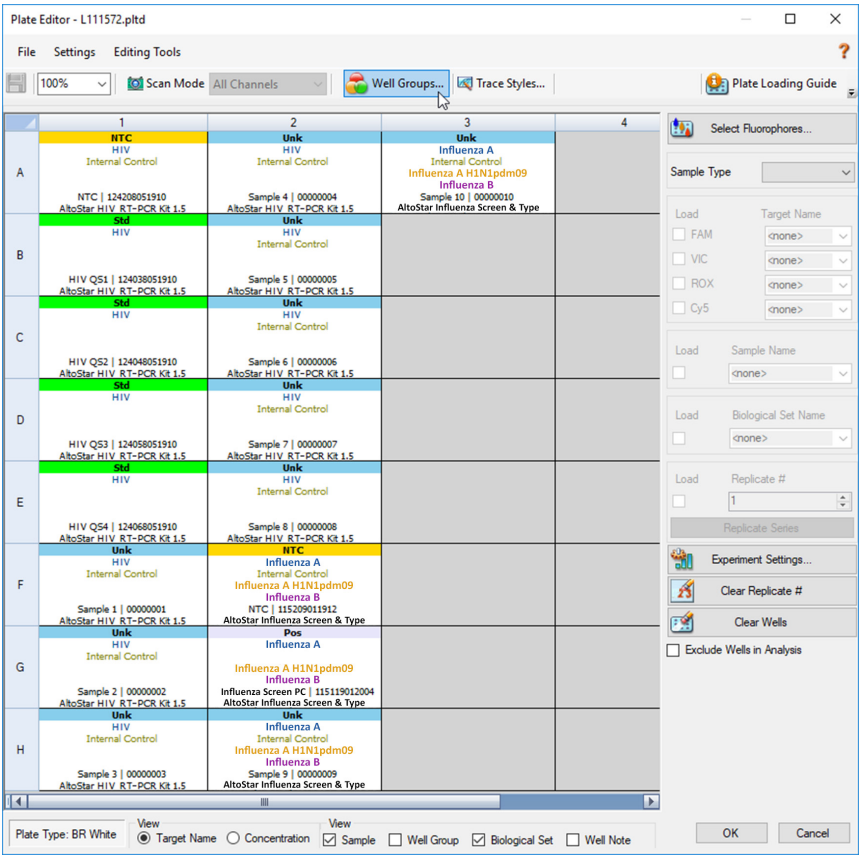

Figure 1: Plate Editor dialog

2. In the Plate Editor dialog click **Well Groups**… in the tool bar. The Well Groups Manager dialog is displayed (see figure 2).
3. Click the **Add** button.
4. Type the name of the first assay in the text box.

5. Select all wells in the PCR plate area that belong to the first assay (see figure 2). The wells belonging to an individual assay can be identified in the Plate Editor dialog by the entry in the **Biological Set** field.

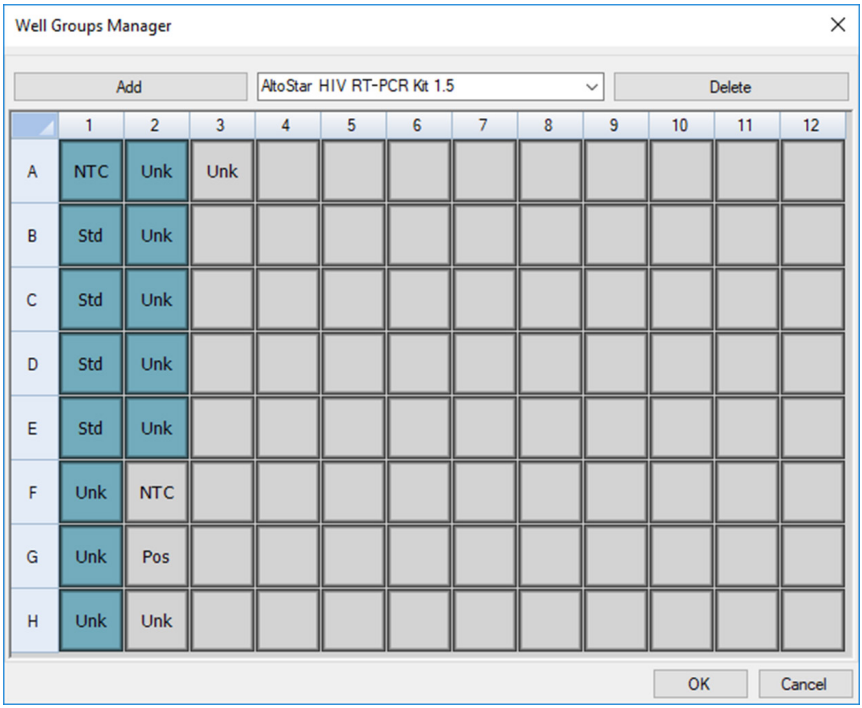

Figure 2: Well Groups Manager dialog

6. Repeat steps 3–5 for all assays on the PCR plate.
7. Confirm well group assignment by clicking **OK**. The Well Groups Manager dialog closes.
8. Close the Plate Editor dialog by clicking **OK**.
9. Confirm to apply the changes by clicking **Yes**.

7.6.7 PCR data analysis

The results of all assays (well groups) on the PCR plate have to be analyzed in the sequence depicted in figure 3.

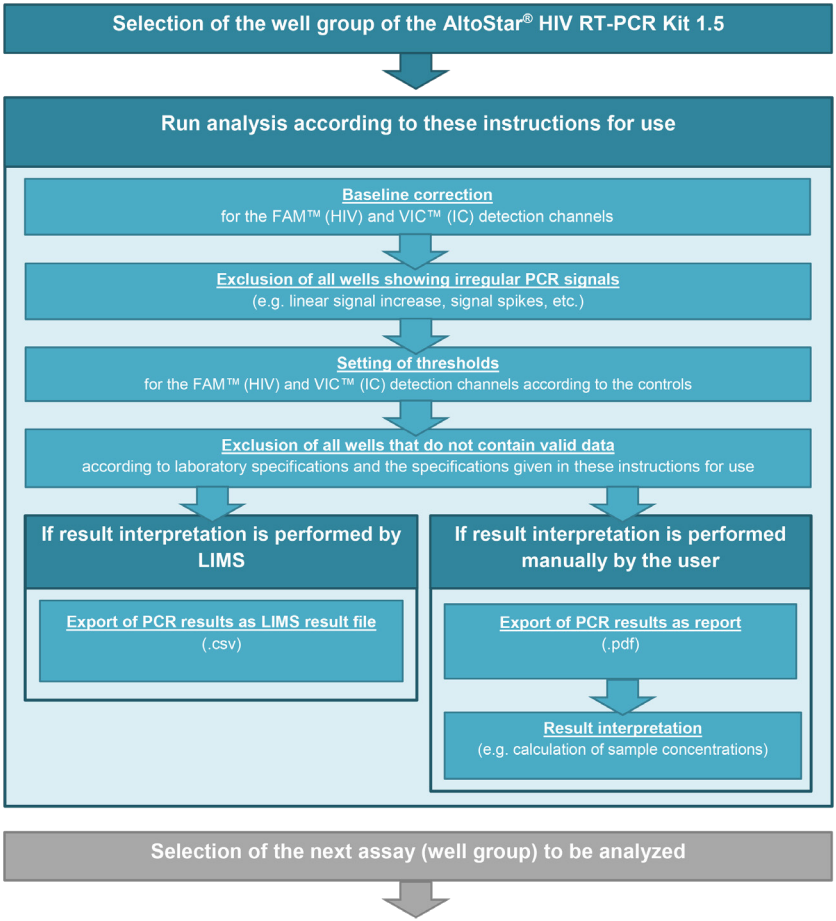

Figure 3: PCR Data Analysis process

In the Data Analysis window ensure to select the **Well Group** of the AltoStar® HIV RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar. Do not use the *"All Wells"* **Well Group**. The selection in figure 4 is used as a general example view.

Before analyzing the results, ensure that the well group of the AltoStar® HIV RT-PCR Kit 1.5 contains all of the AltoStar® HIV RT-PCR Kit 1.5 wells and no wells of other assays.

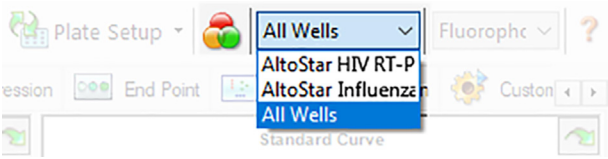

Figure 4: Well Group button and Well Group drop-down menu

NOTE

Combined analysis of more than one assay may lead to incorrect results.

CAUTION

As with any diagnostic test, results shall be interpreted in consideration of all clinical and laboratory findings.

7.6.7.1 Baseline correction

The baseline settings used by the CFX Manager™ Dx software may have to be corrected for individual wells of the assay (**Well Group**) under analysis.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® HIV RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. At the left side of the Data Analysis window tick only the **FAM** checkbox for the HIV target detection channel.

3. In the menu bar of the Data Analysis window click **Settings** → **Baseline Threshold…** to open the Baseline Threshold dialog (see figure 5).
4. Click the ◊ symbol in the **Baseline End** column header once to sort the table by ascending **Baseline End** values.
5. Select all lines that show a **Baseline End** value of 1–9 (see figure 5).

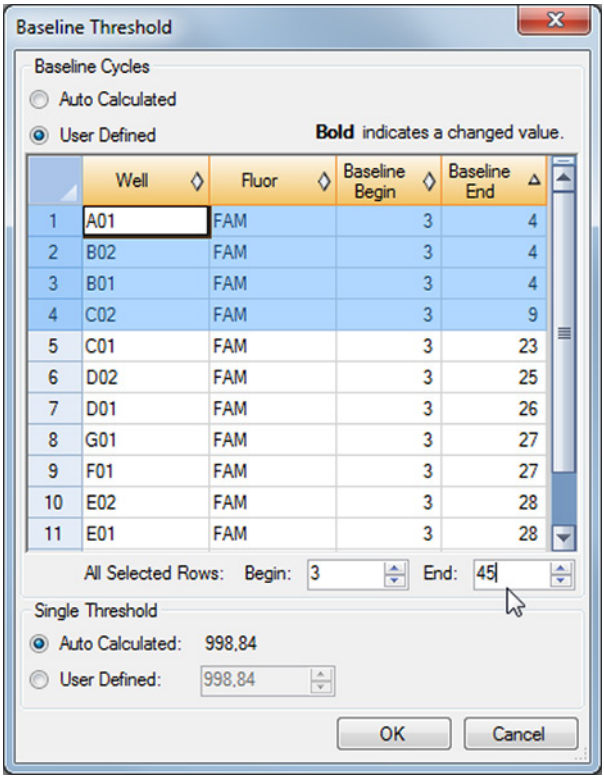

Figure 5: Baseline Threshold dialog

6. Set the value in the **End:** field to 45 for the selected lines (see figure 5).
7. Confirm the settings by clicking **OK**.
8. At the left side of the Data Analysis window untick the **FAM** checkbox and tick only the **VIC** checkbox for the IC target detection channel.
9. Repeat steps 3–7 for the VIC™ (IC) detection channel.

7.6.7.2 Exclusion of irregular PCR signals

Valid results can only be derived from PCR signals that are free of signal artefacts, which may be caused e.g. by impurities or bubbles in the RT-PCR mix. PCR signals that contain signal artefacts have to be excluded by the user.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® HIV RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.

2. Identify wells with irregular PCR signals (linear signal increase, signal spikes, etc.) in any of the FAM™ (HIV target) and the VIC™ (IC) detection channels (see figure 6).

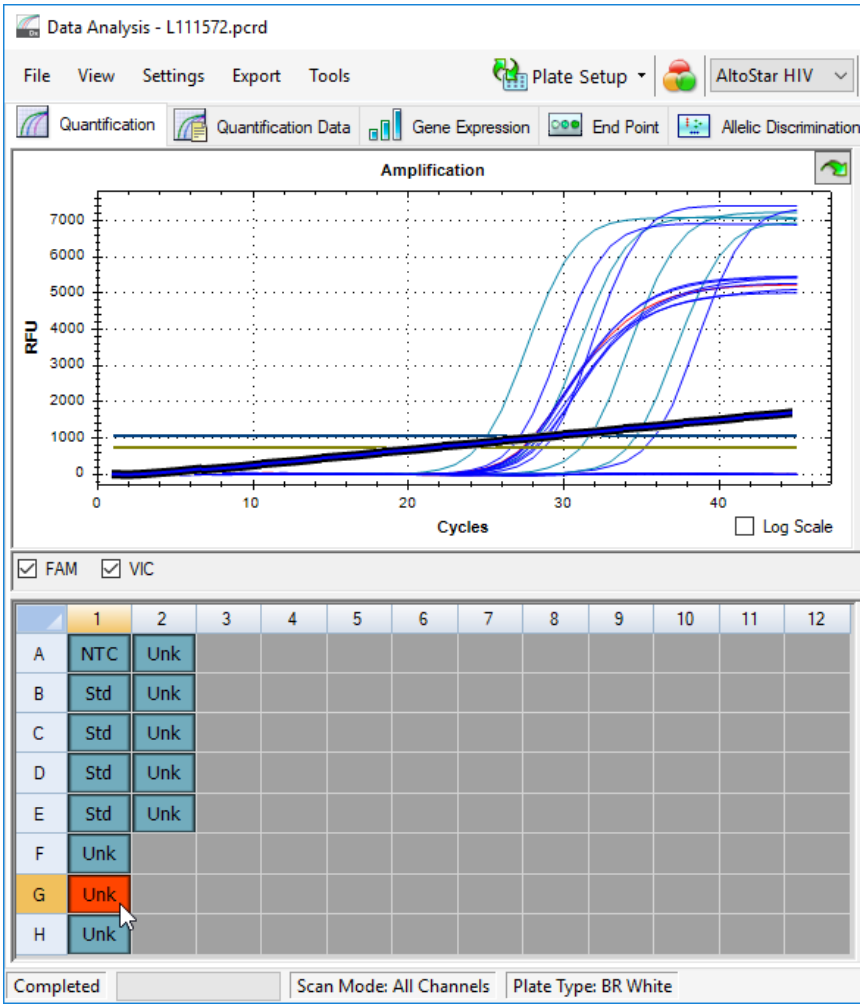

Figure 6: Data Analysis window: irregular PCR signal

3. Click each affected well with the right mouse button and select **Well**... → **Exclude from Analysis** (see figure 7).

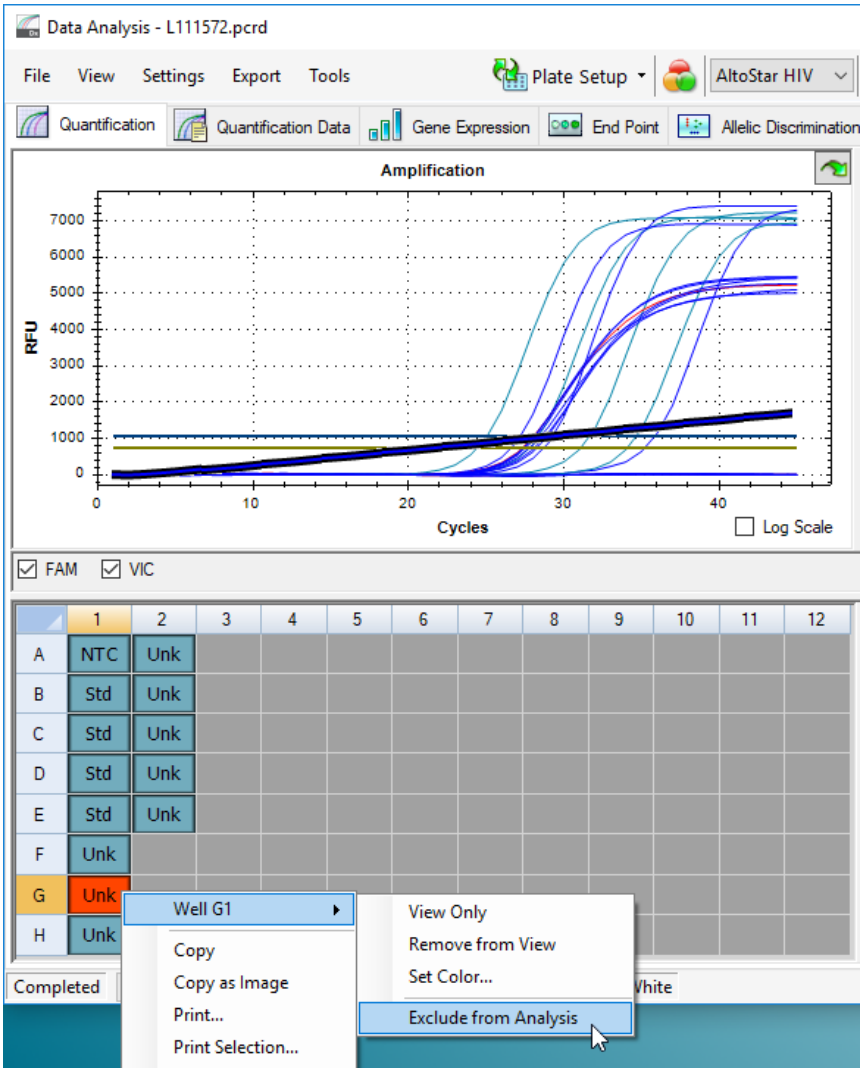

Figure 7: Data Analysis window: exclude well from analysis

4. The selected well is excluded from the analysis. No results will be generated for this well (see figure 8).

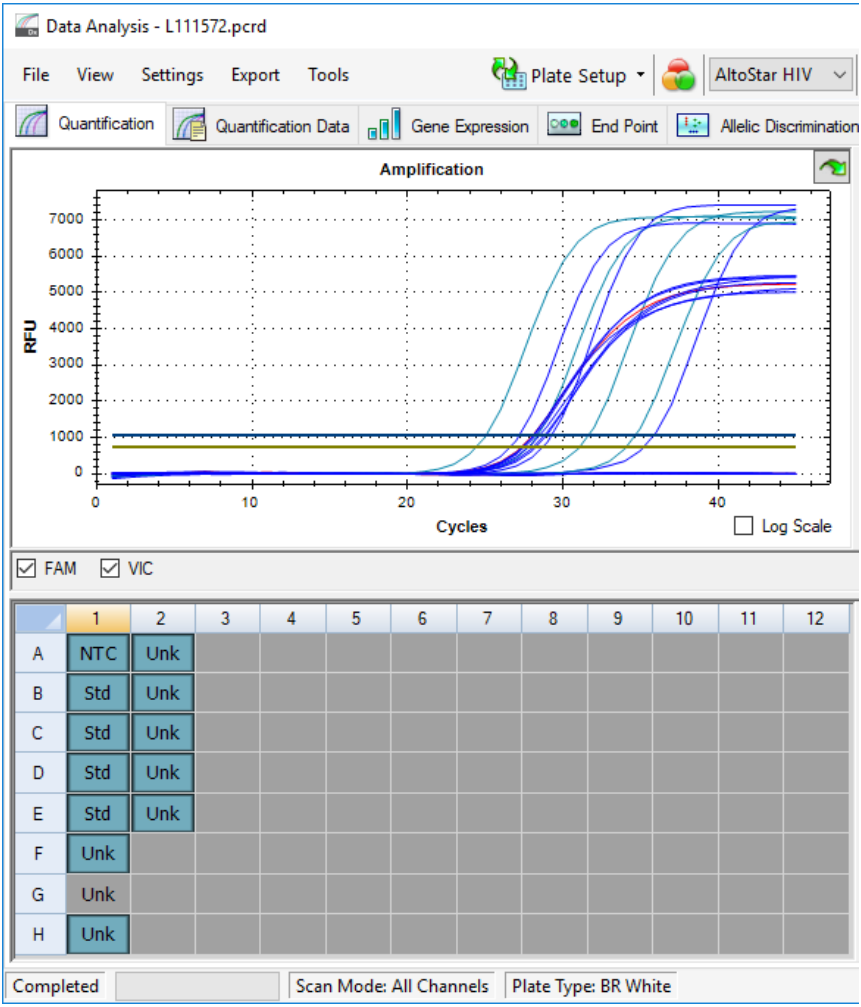

Figure 8: Data Analysis window: excluded well

7.6.7.3 Setting of thresholds

The thresholds for the FAM™ (HIV target) and the VIC™ (IC) detection channels have to be set manually by the user according to the signals of the controls.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® HIV RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.

2. At the left side of the Data Analysis window tick only the **VIC** checkbox for the detection channel of the IC (see figure 9).

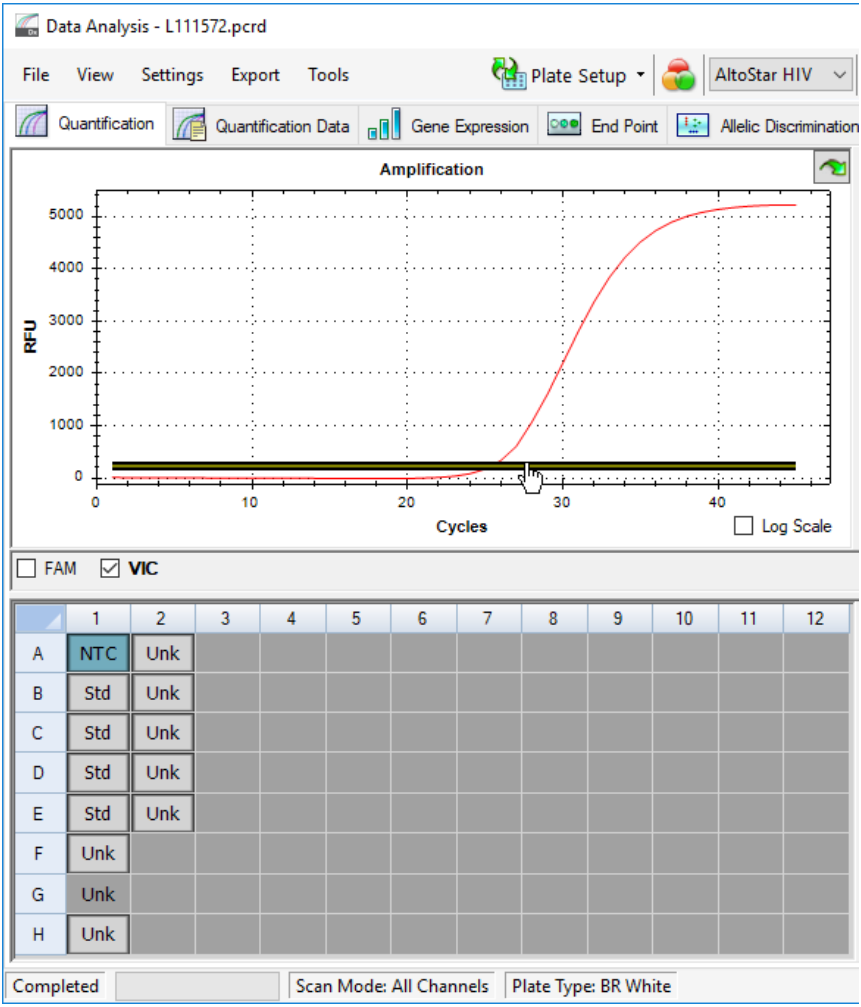

Figure 9: Data Analysis window: setting the VIC™ threshold

3. Select only the NTC well in the plate view of the Data Analysis window (see figure 9).
4. Drag the threshold into the exponential area of the NTC signal (see figure 9).

NOTE

The NTC contains the IC template, which leads to an IC signal in a valid NTC well.

5. At the left side of the Data Analysis window untick the **VIC** checkbox and tick the **FAM** checkbox for the detection channel of the HIV target (see figure 10).

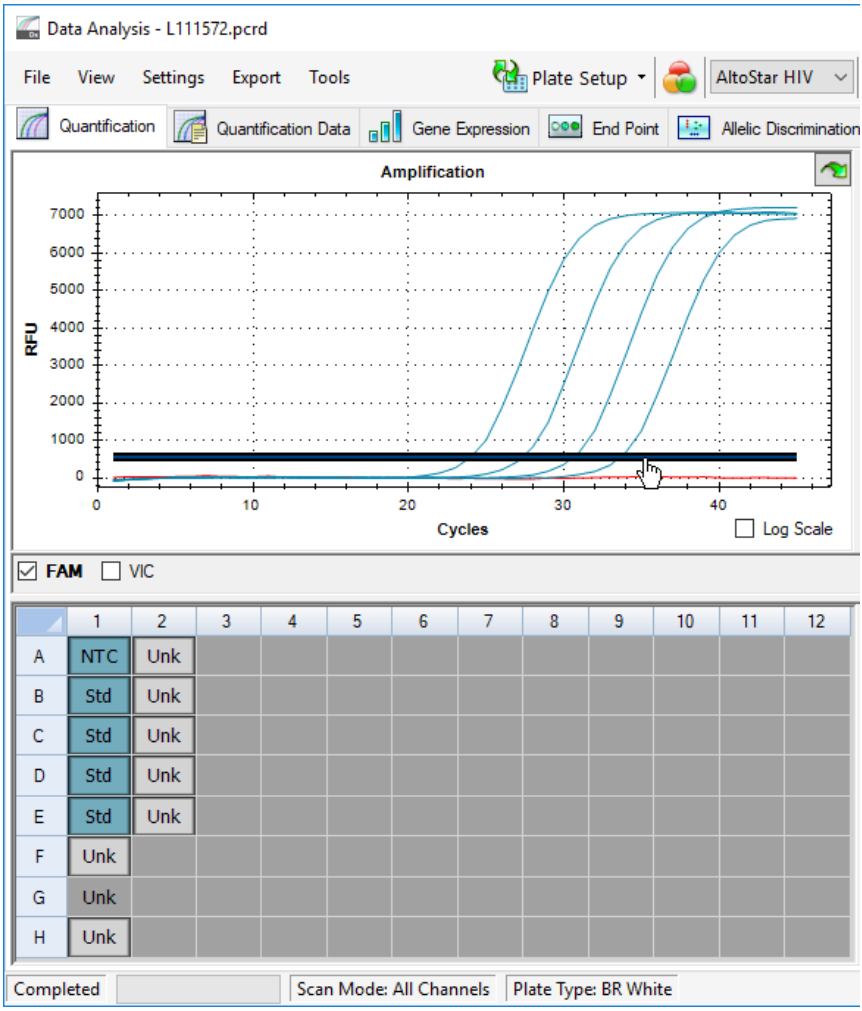

Figure 10: Data Analysis window: setting the FAM™ threshold

6. Select only the wells containing the NTC and the QSs in the plate view of the Data Analysis window (see figure 10).
7. Drag the threshold well above the signal of the NTC into the exponential area of the QSs signals (see figure 10).

7.6.8 Validity of PCR results

7.6.8.1 Exclusion of wells containing invalid data

Wells that do not contain valid data have to be excluded from result generation by the user.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® HIV RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. Identify all wells containing invalid data. A well is invalid if any of the following conditions apply:

    a) The complete run is invalid (see chapter 7.6.8.2 Validity of a diagnostic PCR run).

    b) The well data does not meet the control conditions for a valid result (see chapter 7.6.8.3 Validity of results for a sample).

3. Click each well containing invalid data according to chapters 7.6.8.2 Validity of a diagnostic PCR run to 7.6.8.3 Validity of results for a sample with the right mouse button and select **Well**... → **Exclude from Analysis** (see figures 11 and 12).

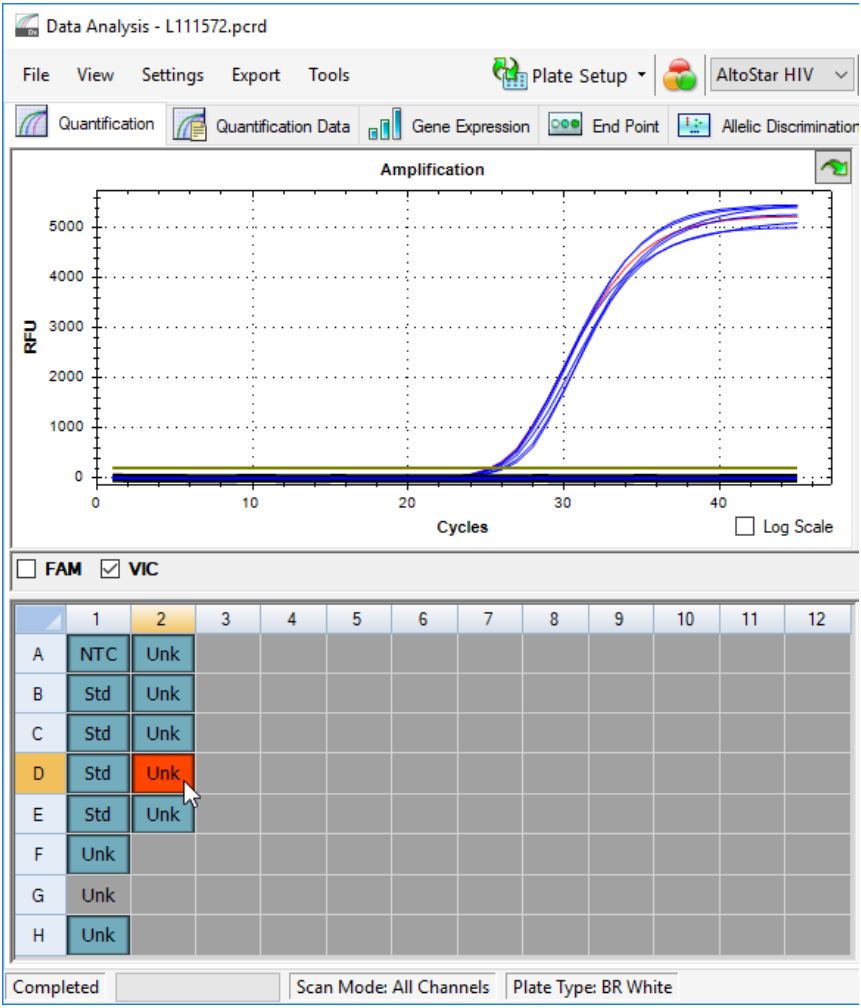

Figure 11: Data Analysis window: invalid well

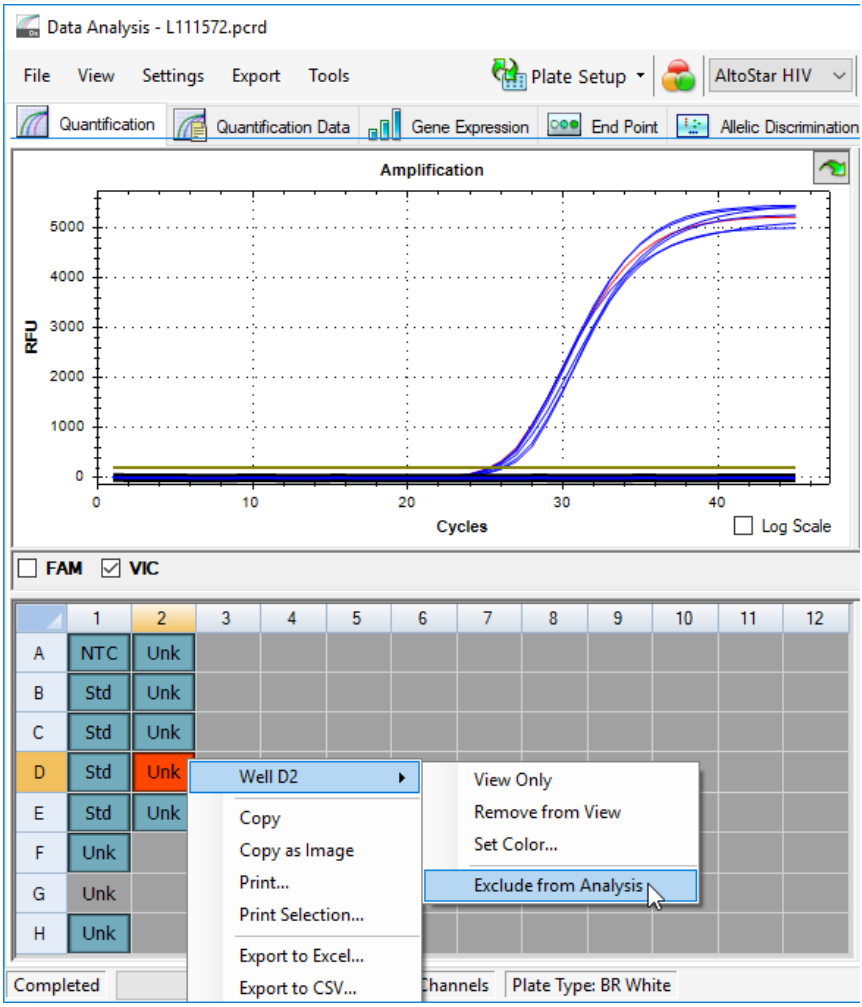

Figure 12: Data Analysis window: exclude invalid well from analysis

The selected well is excluded from the analysis. No results will be generated for this well.

7.6.8.2 Validity of a diagnostic PCR run

A diagnostic PCR run is **valid**, if:

a) the following control conditions are met:

Table 8: Control conditions for a valid PCR run

Control	Detection channel	
	FAM™ (HIV target)	VIC™ (IC)
QS	+	Not applicable
NTC	-	+

and b) the generated standard curve reaches the following control parameter value:

Table 9: Standard curve control parameter

Control parameter	Valid value
R square (R^2)	≥ 0.98

The standard curve's control parameter is displayed below the Standard curve graph in the Data Analysis window (see figure 13).

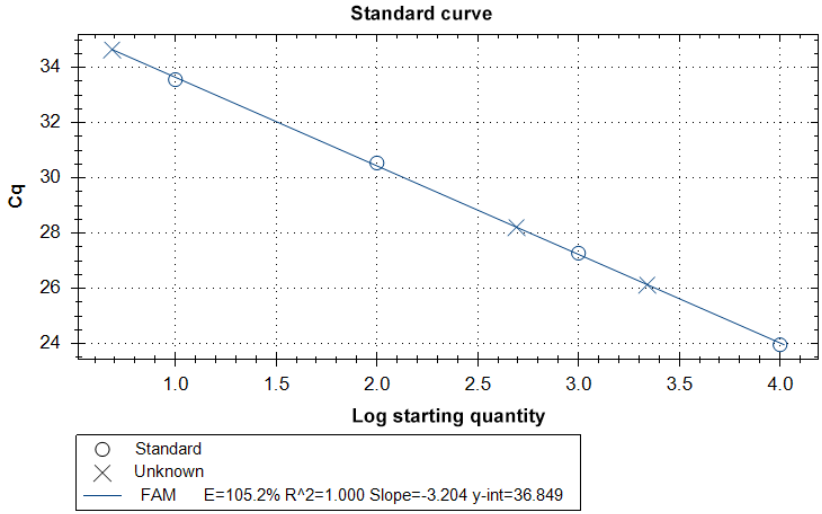

Figure 13: Standard curve data

A diagnostic PCR run is **invalid**, if:

- The run has not been completed.
- Any of the control conditions for a valid diagnostic PCR run are not met.

In case of an invalid diagnostic PCR run, exclude all wells from the analysis and repeat the AltoStar® run starting from the original samples.

7.6.8.3 Validity of results for a sample

The result for an individual sample is **invalid**, if the signals in both the VIC™ (IC) detection channel and the FAM™ (HIV target) detection channel are negative (see table 10). In case of an invalid result for a sample, exclude the well from the analysis and repeat testing from the original sample or collect and test a new sample.

Table 10: Result validity

Detection channel		Result validity
FAM™ (HIV target)	VIC™ (IC)	
+	+	Valid result
+	-	Valid result*
-	+	Valid result
-	-	**Invalid result**

* Detection of the IC is not required when the HIV target is detected. A high HIV RNA load in the sample can lead to a reduced or absent IC signal.

7.6.9 Export of PCR results for automated result interpretation

To make the PCR run results available to a connected LIMS for automated result interpretation they need to be exported in the form of a LIMS result file (.csv).

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® HIV RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. Ensure that all steps of the analysis process (see chapters 7.6.7.1 Baseline correction to 7.6.8.1 Exclusion of wells containing invalid data) have been completed for the well group of the AltoStar® HIV RT-PCR Kit 1.5.
3. In the menu bar of the Data Analysis window click **Export** → **Export All Data Sheets** to open the Browse For Folder dialog.
4. In the Browse For Folder dialog specify the location for the LIMS result files to be generated and click **OK**.

NOTE

The LIMS integration has to be implemented according to the specifications of altona Diagnostics. For information regarding LIMS integration, see chapter 15. Assay protocol for the AltoStar® Connect software and information for LIMS integration and/or contact altona Diagnostics technical support (see chapter 11. Technical support).

NOTE

Saving results of more than one assay (well group) from a PCR run in the same folder leads to replacement of the LIMS result files of the first assay (well group) with the LIMS result files of the second assay (well group). In this case the LIMS result files of the first assay (well group) can be exported again.

7.6.10 Export of PCR results for manual result interpretation

If the results are not passed to a LIMS for automatic result interpretation, the result interpretation has to be performed manually by the user. To this end the analysis results of each assay (well group) need to be exported in the form of a report.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® HIV RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. At the left side of the Data Analysis window tick the **VIC** checkbox as well as the **FAM** checkbox.
3. Ensure that all steps of the analysis process (see chapters 7.6.7.1 Baseline correction to 7.6.8.1 Exclusion of wells containing invalid data) have been completed for the well group of the AltoStar® HIV RT-PCR Kit 1.5.
4. In the menu bar of the Data Analysis window click **Tools** → **Reports**... to open the Report dialog.

5. Ensure that at least the following content is selected for report generation in the upper left part of the Report dialog (see figure 14):

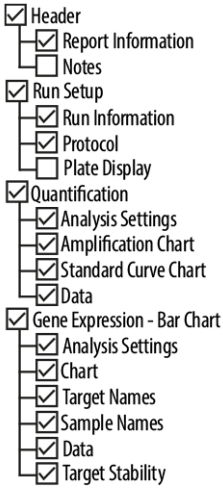

Figure 14: Report dialog

6. Select or deselect additional content of the report by ticking the respective check boxes as required.
7. In the menu bar of the Report dialog click **File** → **Save As**... to open the Save Report dialog.
8. In the Save Report dialog specify the name and location for the report file to be generated and click **Save**.

7.6.10.1 Manual interpretation of results

1. Open the report file generated for the well group of the AltoStar® HIV RT-PCR Kit 1.5 (see chapter 7.6.10 Export of PCR results for manual result interpretation).
2. Refer to the Quantification Data table in the report (see figure 15). The table comprises 2 rows for each **Sample** – one for the **Target** *HIV* and one for the **Target** *Internal Control.*

Quantification Data

Well	Fluor	Target	Content	Sample	Biological Set Name	Cq	Cq Mean	Cq Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean	SQ Std. Dev	Well Note
A01	FAM	HIV	NTC	NTC | 124208051910	AltoStar HIV RT-PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	
A02	FAM	HIV	Unkn	Sample 4 | 00000004	AltoStar HIV RT-PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	Concentration factor: 80.00 IU/ml
B01	FAM	HIV	Std	HIV QS1 | 124038051910	AltoStar HIV RT-PCR Kit 1.5	23.97	23.97	0.000	1.000E+04	4.000	1.00E+04	0.00E+00	
B02	FAM	HIV	Unkn	Sample 5 | 00000005	AltoStar HIV RT-PCR Kit 1.5	34.65	34.65	0.000	4.855E+00	0.686	4.85E+00	0.00E+00	Concentration factor: 80.00 IU/ml
C01	FAM	HIV	Std	HIV QS2 | 124048051910	AltoStar HIV RT-PCR Kit 1.5	27.28	27.28	0.000	1.000E+03	3.000	1.00E+03	0.00E+00	
C02	FAM	HIV	Unkn	Sample 6 | 00000006	AltoStar HIV RT-PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	Concentration factor: 80.00 IU/ml
D01	FAM	HIV	Std	HIV QS3 | 124058051910	AltoStar HIV RT-PCR Kit 1.5	30.54	30.54	0.000	1.000E+02	2.000	1.00E+02	0.00E+00	
E01	FAM	HIV	Std	HIV QS4 | 124068051910	AltoStar HIV RT-PCR Kit 1.5	33.56	33.56	0.000	1.000E+01	1.000	1.00E+01	0.00E+00	
E02	FAM	HIV	Unkn	Sample 8 | 00000008	AltoStar HIV RT-PCR Kit 1.5	28.22	28.22	0.000	4.924E+02	2.692	4.92E+02	0.00E+00	Concentration factor: 80.00 IU/ml
F01	FAM	HIV	Unkn	Sample 1 | 00000001	AltoStar HIV RT-PCR Kit 1.5	26.14	26.14	0.000	2.195E+03	3.341	2.20E+03	0.00E+00	Concentration factor: 80.00 IU/ml
H01	FAM	HIV	Unkn	Sample 3 | 00000003	AltoStar HIV RT-PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	Concentration factor: 80.00 IU/ml
A01	VIC	Internal Control	NTC	NTC | 124208051910	AltoStar HIV RT-PCR Kit 1.5	25.27	25.27	0.000	N/A	N/A	N/A	0.00E+00	
A02	VIC	Internal Control	Unkn	Sample 4 | 00000004	AltoStar HIV RT-PCR Kit 1.5	26.08	26.08	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 80.00 IU/ml
B02	VIC	Internal Control	Unkn	Sample 5 | 00000005	AltoStar HIV RT-PCR Kit 1.5	25.46	25.46	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 80.00 IU/ml
C02	VIC	Internal Control	Unkn	Sample 6 | 00000006	AltoStar HIV RT-PCR Kit 1.5	25.33	25.33	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 80.00 IU/ml
E02	VIC	Internal Control	Unkn	Sample 8 | 00000008	AltoStar HIV RT-PCR Kit 1.5	26.25	26.25	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 80.00 IU/ml
F01	VIC	Internal Control	Unkn	Sample 1 | 00000001	AltoStar HIV RT-PCR Kit 1.5	25.45	25.45	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 80.00 IU/ml
H01	VIC	Internal Control	Unkn	Sample 3 | 00000003	AltoStar HIV RT-PCR Kit 1.5	25.70	25.70	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 80.00 IU/ml

Figure 15: Report: Quantification Data

Results are marked by a *Concentration factor* in the **Well Note** column of the Quantification Data table (see figure 15).

3. Refer to the **Starting Quantity (SQ)** column for the concentration of the HIV target measured in the eluate of the respective **Sample**. To calculate the result for the original patient sample, the **Starting Quantity (SQ)** value has to be multiplied by the respective *Concentration factor* (including the unit) by the user.

4. Refer to table 11 for interpretation of results.

Table 11: Result interpretation

Starting Quantity (SQ) of the HIV target	Result interpretation
> 0	HIV specific RNA detected. Multiply the **Starting Quantity (SQ)** value by the *Concentration factor* in the **Well Note** column (including the unit) to calculate the concentration of the original patient sample.
N/A	No HIV specific RNA detected. Sample does not contain detectable amounts of HIV specific RNA.

NOTE

Results obtained after multiplication by the concentration factor indicate the viral load (sample) in IU/ml. For conversion to viral load (sample) in copies/ml, use the following conversion factor: 0.42 copies/IU (1 IU = 0.42 copies).

8. Performance data

The performance of the AltoStar® HIV RT-PCR Kit 1.5 was evaluated using the 4th WHO International Standard for HIV-1 RNA (NIBSC code: 16/194; subtype B) provided by the NIBSC (National Institute for Biological Standards and Control) and HIV commercially available virus material calibrated against the WHO International Standard.

8.1 Plasma

8.1.1 Analytical sensitivity

For the determination of the limit of detection (LoD) a dilution series of the 4th WHO International Standard for HIV-1 RNA (NIBSC code: 16/194; subtype B) in EDTA plasma ranging from 5.00E+02 to 1.00E+00 IU/ml was generated.

Each dilution was tested in 8 replicates in 3 different runs (total n = 24 per dilution) using combinations of:

- 3 AltoStar® HIV RT-PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 12: PCR results used for the calculation of the analytical sensitivity of the AltoStar® HIV RT-PCR Kit 1.5

Concentration [IU/ml]	N [total]	N [positive]	Hit rate [%]
5.00E+02	24	24	100
2.00E+02	24	24	100
1.00E+02	24	24	100
8.00E+01	24	24	100
5.00E+01	24	23	96
2.00E+01	24	22	92
1.00E+01	24	15	63
5.00E+00	24	6	25
1.00E+00	24	0	0

The LoD of the AltoStar® HIV RT-PCR Kit 1.5 for the detection of HIV in EDTA plasma is 30 IU/ml (95 % confidence interval: 21–54 IU/ml).

The LoD for the HIV subtypes contained in group M (A, C, D, E, F, G, H, AG), group N, as well as in group O was confirmed according to the protocol described in CLSI guideline EP17-A2 (“Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition”).

For each HIV subtype negative EDTA plasma was spiked with HIV to a final concentration of the LoD value (30 IU/ml) determined for the 4th WHO International Standard for HIV-1 RNA (NIBSC code: 16/194; subtype B).

Table 13: Confirmation of the analytical sensitivity for HIV subtypes

HIV subtype	N [total]	N [positive]	Hit rate [%]
A	60	56	93
C	60	55	92
D	60	56	93
E	60	58	97
F	60	54	90
G	60	60	100
H	60	54	90
AG	60	55	92
Group N	60	60	100
Group O	59*	55	93

* One sample was not processed.

The results confirmed a LoD of at least 30 IU/ml for HIV subtypes contained in group M (A, C, D, E, F, G, H, AG), group N, as well as in group O.

8.1.2 Analytical specificity

The analytical specificity of the AltoStar® HIV RT-PCR Kit 1.5 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant HIV genotypes will be detected.

For the verification of the analytical specificity of the AltoStar® HIV RT-PCR Kit 1.5 the following experiments were performed (see chapters 8.1.2.1 Negative samples to 8.1.2.3 Cross-reactivity).

8.1.2.1 Negative samples

103 HIV negative EDTA plasma samples from individual donors were tested with the AltoStar® HIV RT-PCR Kit 1.5. All (103 out of 103) samples were tested negative for HIV specific RNA and positive for the IC. The analytical specificity of the AltoStar® HIV RT-PCR Kit 1.5 for EDTA plasma samples is ≥ 99 %.

8.1.2.2 Interfering substances

To evaluate the influence of potentially interfering endogenous and exogenous substances on the performance of the AltoStar® HIV RT-PCR Kit 1.5 selected substances were spiked in EDTA plasma samples. These plasma samples contained HIV in a concentration of the 3 x LoD (9.00E+01 IU/ml), 5.00E+03 IU/ml and no HIV, respectively.

Results obtained for samples containing potentially interfering substances were compared to results generated for EDTA plasma samples containing no spiked interferent. Each sample was processed in 3 replicates.

No interference was observed for samples containing elevated levels of:

- Endogenous substances
  - Bilirubin
  - Hemoglobin
  - Human genomic DNA
  - Human serum albumin
  - Triglycerides
- Exogenous substances
  - Abacavir
  - Atazanavir

- Efavirenz
- Emtricitabin
- Lamivudine
- Raltegravir
- Tenofovir

Additionally, EDTA plasma samples from patients suffering from autoimmune diseases (systemic lupus erythematosus and rheumatoid arthritis) were tested. No interference in terms of specificity, sensitivity and reliable quantification was observed.

CAUTION

The presence of PCR inhibitors (e.g. heparin) could cause false negative or invalid results.

8.1.2.3 Cross-reactivity

The analytical specificity of the AltoStar® HIV RT-PCR Kit 1.5 with respect to cross-reactivity with other pathogens than HIV was evaluated by testing:

- Viruses related to HIV
- Viruses causing similar symptoms as an infection with HIV
- Viruses likely to be present in patients suffering from an HIV infection

The AltoStar® HIV RT-PCR Kit 1.5 did not cross-react with any of the following pathogens:

- *Candida abicans*
- Cytomegalovirus (CMV)
- Hepatitis A virus (HAV)
- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- Herpes simplex virus 1 (HSV-1)
- Herpes simplex virus 2 (HSV-2)
- Human immunodeficiency virus 2 (HIV-2)
- Human T-lymphotropic virus-I (HTLV-I)
- Human T-lymphotropic virus-II (HTLV-II)
- Parovirus B19

CAUTION

In case the sample contains other pathogens than HIV, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.

8.1.3 Linear range

For the determination of the linear range of the AltoStar® HIV RT-PCR Kit 1.5 a dilution series of HIV in EDTA plasma ranging from 1.00E+07 to 1.00E+02 IU/ml was tested:

- Dilutions with a concentration between 1.00E+07 and 1.00E+05 IU/ml were tested in 4 replicates.
- Dilutions with a concentration between 1.00E+04 and 1.00E+02 IU/ml were tested in 8 replicates.

Analysis was performed based on a polynomial regression.

The linear range of the AltoStar® HIV RT-PCR Kit 1.5 for the quantification of HIV in EDTA plasma is 1.00E+02–1.00E+07 IU/ml. A graphical representation of the data is shown in figure 16.

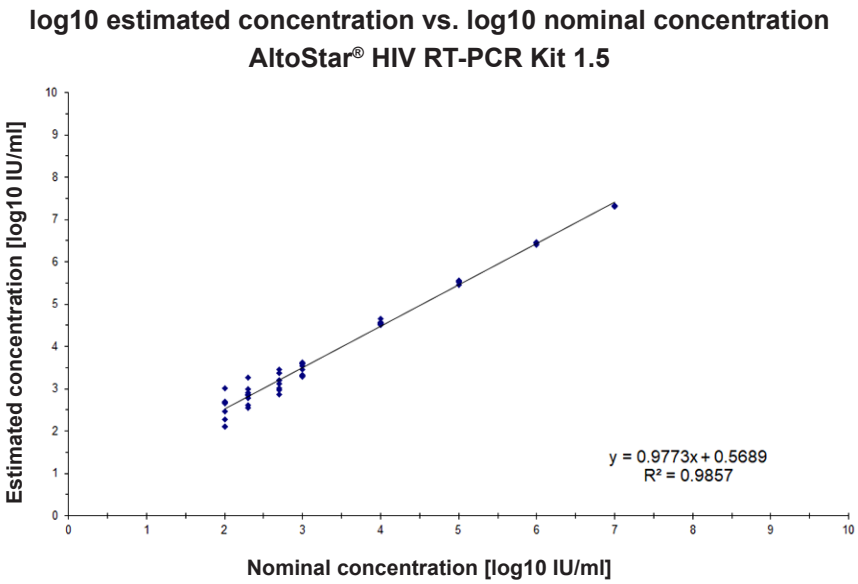

Figure 16: Linear regression analysis of the AltoStar® HIV RT-PCR Kit 1.5 with EDTA plasma samples

8.1.4 Precision

Precision of the AltoStar® HIV RT-PCR Kit 1.5 was evaluated using a panel consisting of:

- 1 HIV high positive (5.00E+03 IU/ml) EDTA plasma sample
- 1 HIV low positive [5.00E+02 IU/ml (5 x lower limit of quantitation (LLoQ))] EDTA plasma sample
- 1 HIV negative EDTA plasma sample

Each panel member was tested in at least 4 replicates per run.

5 runs were performed on 5 different days using combinations of:

- 3 AltoStar® HIV RT-PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Repeatability (intra-run variability), inter-lot variability and reproducibility (total variability) were determined based on:

- Quantification values for the HIV high and low positive samples (see table 14)
- Threshold cycle (C_q) values for the IC in the HIV negative samples (see table 15)

Table 14: Precision data (CV % log10 quantification data) for HIV high and low positive EDTA plasma samples

	HIV high positive sample (5.00E+03 IU/ml)	HIV low positive sample (5.00E+02 IU/ml)
Intra-run variability	0.35–0.89	1.82–3.21
Inter-lot variability	1.49	2.54
Total variability	1.25	2.92

Table 15: Precision data (CV % C_q values) for the IC in HIV negative EDTA plasma samples

	IC
Intra-run variability	0.27–2.94
Inter-lot variability	2.40
Total variability	3.13

In addition, site-to-site variability was assessed by testing a sample panel at 3 different laboratories. The sample panel comprised HIV positive EDTA plasma samples at 3 different concentrations [5.00E+03 IU/ml, 5.00E+02 IU/ml (5 x LLoQ) and 9.00E+01 IU/ml (3 x LoD)] as well as HIV negative samples. The results obtained for the 5.00E+03 IU/ml and 5.00E+02 IU/ml sample are summarized in table 16.

Table 16: Precision data (CV % log10 quantification data) site-to-site variability

	HIV high positive sample (5.00E+03 IU/ml)	HIV low positive sample (5.00E+02 IU/ml)
Site-to-site variability	0.97	2.71

8.1.5 Total failure rate

The robustness of the AltoStar® HIV RT-PCR Kit 1.5 was assessed by testing 108 HIV negative EDTA plasma samples from individual donors spiked with HIV to a final concentration of the 3 x LoD (9.00E+01 IU/ml). 99.1 % (107 out of 108) of the samples were tested positive in the HIV specific fluorescence detection channel (FAM™).

8.1.6 Carry over

Carry over is mostly a workflow dependent risk and independent of the PCR assay used. For the AltoStar® Workflow the AltoStar® Parvovirus B19 PCR Kit 1.5 was used as exemplary model. Potential cross-contamination through carry over from high positive samples was evaluated by testing alternating parvovirus B19 high positive (1.00E+07 IU/ml) and negative samples (n = 23 each per run; 5 runs) with the AltoStar® Parvovirus B19 PCR Kit 1.5. No carry over was observed, i.e. all parvovirus B19 negative samples were tested negative.

8.1.7 Clinical performance

The AltoStar® HIV RT-PCR Kit 1.5 was evaluated in a comparative study with the CE-marked cobas® HIV-1 test (Roche). Retrospectively, 244 EDTA plasma samples from routine HIV monitoring were tested in parallel with the CE-marked cobas® HIV-1 test (Roche) and the AltoStar® HIV RT-PCR Kit 1.5.

The cobas® HIV-1 test (Roche) was used in combination with the cobas® 6800 system (Roche).

The AltoStar® HIV RT-PCR Kit 1.5 was used in combination with the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 on the AltoStar® AM16 and the CFX96™ DW Dx.

For the analysis of diagnostic sensitivity and specificity 238 valid samples were used. Results are shown in table 17.

Table 17: Results of the evaluation of the diagnostic sensitivity and specificity for HIV in EDTA plasma samples

		cobas® HIV-1 test (Roche)	
		POSITIVE	NEGATIVE
AltoStar® HIV RT-PCR Kit 1.5	POSITIVE	140	1
	NEGATIVE	6	91

The diagnostic sensitivity and specificity of the AltoStar® HIV RT-PCR Kit 1.5 compared to the cobas® HIV-1 test (Roche) were 96 % (confidence interval 91.3 %–98.5%) and 99 % (confidence interval 94.1 %–99.9 %), respectively.

For the quantitative correlation samples tested negative with one or both assays and samples with a quantitative result below the LLoQ of one or both assays were excluded.

The results of the remaining 116 samples were used for the quantitative correlation by linear regression analysis (see figure 17).

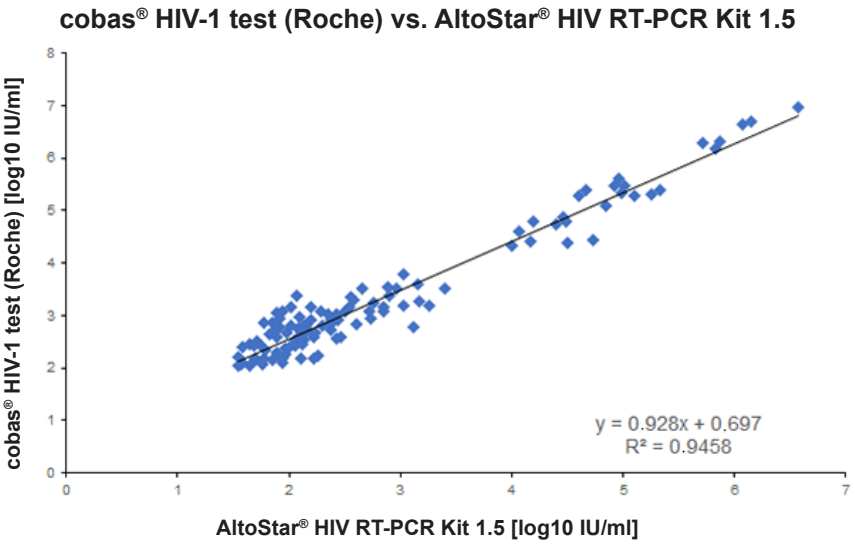

Figure 17: Linear regression analysis of the results obtained with the cobas® HIV-1 test (Roche) (reference) and the AltoStar® HIV RT-PCR Kit 1.5

There was very good correlation between the quantitative results obtained with the AltoStar® HIV RT-PCR Kit 1.5 and the cobas® HIV-1 test (Roche) [correlation coefficient R = 0.97 (R^2 = 0.95)].

9. Disposal

Dispose of hazardous and biological waste in compliance with local and national regulations. Leftover product components and waste should not be allowed to enter sewage, water courses or the soil.

CAUTION

Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

CAUTION

Disposal of hazardous and biological waste shall comply with local and national regulations to avoid environmental contamination.

NOTE

The PCR plate must be disposed of in a sealed state since the PCR Plate Sealing Foil cannot be removed.

10. Quality control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of AltoStar® HIV RT-PCR Kit 1.5 is tested against predetermined specifications to ensure consistent product quality.

11. Technical support

For customer support, contact altona Diagnostics technical support:

e-mail: **support@altona-diagnostics.com**

phone: **+49-(0)40-5480676-0**

NOTE

Any serious incident that has occurred in relation to this product shall be reported to altona Diagnostics and the competent authority of your country.

12. Literature

[1] Zhou H, et al. “Genome-Scale RNAi Screen for Host Factors Required for HIV Replication”. Cell Host & Microbe. 2008 Nov 13: Vol. 4, Issue 5, pp. 495-504.

[2] Rambaut A, et al. “The causes and consequences of HIV evolution”. Nature Reviews Genetics. 2004 Jan 4; Vol. 5, Issue 1, pp. 52-61.

[3] Weiss RA. “How does HIV cause AIDS?” Science. 1993 May 28: Vol. 260, Issue 5112, pp. 1273-1279.

[4] Joint United Nations Programme on AIDS/HIV (UNAIDS). UN AIDS Data 2019. Available at https://www.unaids.org/sites/default/files/media_asset/2019-UNAIDS-data_en.pdf (2019), accessed September 26, 2019.

[5] Eisinger R et al. “HIV viral load and transmissibility of HIV infection: Undetectable equals untransmittable". JAMA. 2019 Feb 5, Vol. 321, Issue 5, pp. 451-452.

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[7] Deeks SG, et al. “The end of AIDS: HIV infection as a chronic disease”. Lancet. 2013 Nov 2, Vol. 382, Issue 9903, pp. 1525-1533.

13. Trademarks and disclaimers

4s3™ (4titude); AltoStar® (altona Diagnostics); CFX96™, CFX Manager™ (Bio-Rad); LOINC® (Regenstrief Institute, Inc.); cobas® (Roche); FAM™, VIC™ (Thermo Fisher Scientific).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The AltoStar® HIV RT-PCR Kit 1.5 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

14. Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
GTIN	Global Trade Item Number
LOT	Batch code
CONT	Content
CAP	Cap color
REF	Catalogue number
NUM	Number
COMP	Component
	Consult instructions for use
	Contains sufficient for "n" tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
MAT	Material number
	Version
	Note

Symbol	Explanation
	Contains biological material of animal origin

15. Assay protocol for the AltoStar® Connect software and information for LIMS integration

The 2D barcode in figure 18 is to be used for installing the latest assay protocol for use of the AltoStar® HIV RT-PCR Kit 1.5 on the AltoStar® AM16. The barcode can only be scanned in printed form. You can scan the barcode directly from the manual or print it on a separate sheet. Please note that the size of the print affects the scannability of the barcode. Make sure to scale the size to 100 %. For scanning, aim the scanner to the red line on the barcode. For details on management of assay protocols, please refer to the respective chapter in the instructions for use of the AltoStar® Connect software. For information for LIMS integration, see table 19.

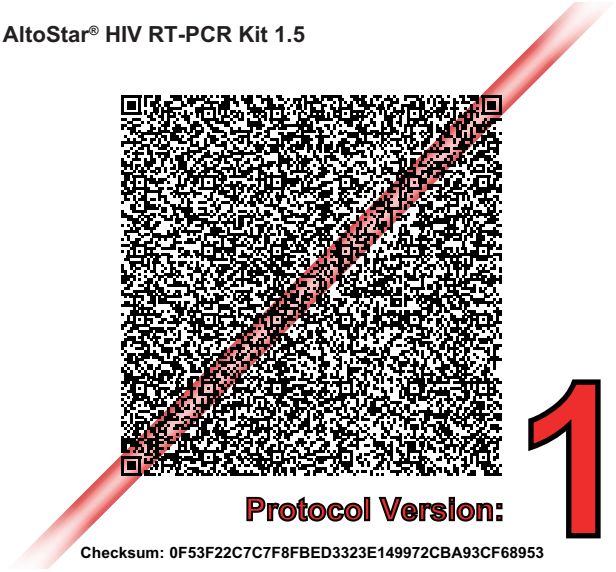

Figure 18: Assay protocol barcode for the AltoStar® HIV RT-PCR Kit 1.5

Table 18: Changelog for the assay protocol

Protocol version	Release updates
1	Initial version

Table 19: Information for LIMS integration

Usage	Data
Test order (LIMS → AltoStar® AM16)	AltoStar® HIV RT-PCR Kit 1.5
Test result (CFX96™ DW Dx → LIMS) unit	IU/ml
Test result (CFX96™ DW Dx → LIMS) channel 1	HIV
Test result (CFX96™ DW Dx → LIMS) channel 2	Internal Control

For LOINC® (Logical Observation Identifiers Names and Codes) please refer to the altona Diagnostics GmbH website (www.altona-diagnostics.com) or contact altona Diagnostics technical support (see chapter 11. Technical support).

16. Revision history

Table 20: Revision history

Identifier	Date of issue [month/year]	Modifications
MAN-AS0221510-EN-S03	10/2021	• adaption of a new structure for the whole instructions for use • adaption of the intended use in chapter 2 • adaption of table 3 “Required material and devices” in chapter 7.4 • addition of two notes in chapters 5.2.2 and 7.6.10.1 giving information on the conversion factor (1 IU = 0.42 copies) • adaption of the description of the sealing process using the new PCR plate sealing foil with the imprint “THIS SIDE UP” and an additional plate sealer in chapter 7.6.5 • addition of the symbol “contains biological material of animal origin” in chapter 14 • introduction of an assay protocol barcode in the new chapter 15
MAN-AS0221510-EN-S04	02/2022	• adaption of the intended use in chapter 2 • specification of the first sentence in the product description in chapter 5

always a drop ahead.

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