altona
DIAGNOSTICS

Instructions for Use

AltoStar®
SARS-CoV-2 RT-PCR Kit 1.5

09/2020   EN

AltoStar®

AltoStar®

SARS-CoV-2 RT-PCR Kit 1.5

For research use only!

(RUO)

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Content

1. About these Instructions for Use

Throughout this manual, the term NOTE has the following meaning:

> **NOTE**
>
> **i** Information is given to the user that is useful but not essential to the task at hand.

2. Application

The AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific RNA.

For research use only (RUO)! Not for use in diagnostic procedures.

3. Kit Content

The AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 contains the following components:

Table 1: Kit Components

Lid Color	AltoStar® SARS-CoV-2 1.5 Component	Number of Tubes	Nominal Volume [µl/Tube]
Blue	Master A	8	60
Purple	Master B	8	180
Red	Positive Control (E gene + S gene)	2	250
White	NTC*	2	250

* No Template Control

The AltoStar® SARS-CoV-2 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 please check the product and its components for:

- Integrity
- Completeness with respect to number, type and filling
- Correct labelling
- 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 10 Technical Assistance).

4. Storage and Handling

All reagents included in the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 are ready-to-use solutions.

4.1 Storage

All components of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 must be stored between -25 °C and -15 °C upon arrival.

NOTE
i Improper storage conditions may lead to a compromised product performance.

NOTE
i Do not use product components beyond the expiration date printed on the component label.

4.2 Handling

> **NOTE**
>
> ℹ
>
> Improper handling of product components and samples may lead to contamination causing incorrect results.
>
> - Do not interchange vial or bottle caps, as cross-contamination may occur.
> - To minimize the risk of carryover contamination 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 wear disposable gloves.
> - Do not open the PCR Plates post amplification to avoid contamination with amplicons.

> **NOTE**
>
> ℹ
>
> Only components of the same kit lot are compatible.

4.2.1 Positive Control and NTC

1. Discard the lids of the Positive Control and NTC tubes at each use and use new lids to avoid contamination of the reagents.

2. After use close the Positive Control and NTC tubes with new lids and freeze them immediately.

5. Product Description

The AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific RNA. It includes two different amplification and detection systems. One primer and probe set enables the detection of an E gene sequence, which is specific for lineage B-betacoronavirus (B-βCoV; including SARS-CoV-2), whereas a second primer and probe set specifically amplifies and detects a SARS-CoV-2 specific sequence of the S gene.

The AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 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 E gene and S gene specific target sequences and fluorescently labelled target specific probes for the detection of the amplified DNA.

In addition to the E gene and S gene RNA specific amplification and detective system the assay includes oligonucleotides for the amplification and detection of the IC (AltoStar® Internal Control 1.5). The IC is automatically added at the beginning of the nucleic acid purification procedure on the AltoStar® AM16. For details refer to the Instructions for Use of the AltoStar® Internal Control 1.5.

Probes specific for E gene RNA are labelled with the fluorophore FAM™ and probes specific for S gene RNA are labelled with the fluorophore Cy5. The probe specific for the IC is labelled with a fluorophore detectable in the VIC™ channel.

Using probes linked to distinguishable dyes enables the parallel detection of E gene and S gene specific RNA and the Internal Control in corresponding detector channels of the CFX96™ DW Dx.

5.1 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, target detection of E gene and S gene specific RNA and of the IC (AltoStar® Internal Control 1.5) in one reaction setup.

5.2 Positive Control

The Positive Control (E gene + S gene) contains standardized concentrations of lineage B betacoronavirus (lineage B-βCoV) and SARS-CoV-2 specific RNA. It is used to verify the functionality of the lineage B-βCoV and SARS-CoV-2 specific amplification and detection systems included in the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5.

5.3 No Template Control

The No Template Control (NTC) contains no lineage B-βCoV and SARS-CoV-2 specific RNA but does contain the Internal Control template. The NTC is used as negative control for the lineage B-βCoV and SARS-CoV-2 RNA specific real-time RT-PCR and indicates possible contamination of Master A and Master B.

5.4 AltoStar® Workflow

The AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 is intended for use within the AltoStar® Workflow. The AltoStar® 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. PCR Setup Run on the AltoStar® AM16 using the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5.
4. Real-time RT-PCR Run on a CFX96™ DW Dx.

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 assays in parallel as the available eluate allows.

> **NOTE**
>
> **i** Assays with differing PCR temperature profiles are automatically sorted to separate PCR Plates.

6. Sample Preparation

6.1 Sample Volume

The AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 has been developed for nucleic acid purifications from a sample volume of 500 µl. Additional sample volume has to be provided to account for the dead volume of the sample tube used (see chapter 6.2 Sample Tubes).

6.2 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.

6.3 Sample Barcodes

For automated sample identification by the AltoStar® AM16 all sample tubes must be labelled with a suitable barcode. For details refer to the Instructions for Use of the AltoStar® Purification Kit 1.5.

7. Material and Devices Required, but not Provided

The material and devices shown in Table 2 must be ordered from altona Diagnostics GmbH.

Table   2: Required material and devices

Material Name	Description	Order No.	Shipping Unit
AltoStar® AM16	AltoStar® Automation System AM16	806160	1
AltoStar® Connect software	AltoStar® Connect software (Version 1.6.16 or higher)	911275	1
CFX96™ DW Dx	CFX96™ Deep Well Dx System* (Bio-Rad) with CFX Manager™ Dx software (Version 3.1)	DT16	1
AltoStar® Purification Kit 1.5	AltoStar® Purification Kit 1.5	PK15-16	1
AltoStar® Internal Control 1.5	AltoStar® Internal Control 1.5	IC15-16	1
PCR Plate	Hard-Shell® PCR Plate 96-Well, Low-Profile, Semi-Skirted, Clear shell, White well - barcoded	VK000005	25
AltoStar® PCR Plate Sealing Foil	AltoStar® PCR Plate Sealing Foil with cut corner A1 and 10 mm sides (SP-0235)	VK000006	100
1000 µl CO-RE Tips	CO-RE Tips, 8 x 480 High Volume Tips (1000 µl) with Filters	VK000007	3840
300 µl CO-RE Tips	CO-RE Tips, 12 x 480 Standard Volume Tips (300 µl) with Filters	VK000008	5760
Pooling Tube	Tube 5 ml, 92 x 15.3 mm (round), PP, with barcode	VK000002	1000
Waste Bag	Sterilbag, Bag Type 60 - Autoclave 134 °C	VK000009	500
Screw Cap - red (cap for QS1 - QS4)	Screw cap for micro tubes, red	VK000012	5000

Material Name	Description	Order No.	Shipping Unit
Screw Cap - blue (cap for Master A)	Screw cap for micro tubes, blue	VK000013	5000
Screw Cap - purple (cap for Master B)	Screw cap for micro tubes, purple	VK000015	5000
Screw Cap - white (cap for NTC)	Screw cap for micro tubes, white	VK000016	5000

* "CFX96™ Deep Well Dx System" is the new brand name for the IVD version of the CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad).

NOTE

i   Do not use other materials or devices than specified in these Instructions for Use.

Table 3: Additional laboratory material and devices

Material	Description	Order No.	Shipping Unit
Plate Sealer	e.g. AltoStar® Plate Sealer	VK000023	1

General material and devices:

- Vortex mixer
- Powder-free gloves (disposable)
- Centrifuge for centrifugation of PCR kit reagents
- Centrifuge for centrifugation of PCR plates

8. Procedure

8.1 Overview of the AltoStar® Workflow

The steps of the AltoStar® Workflow are summarized in Table 4.

Table   4: 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 LIMS, • Select assays for the samples unless already imported from LIMS, • 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 chapter 8.6.1 Sample Preparation.
	• 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 ten 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.
	• If the associated PCR Setup Run is not started right away, seal the Eluate Plate with the AltoStar® Eluate Plate Sealing Foil and store at 2 °C - 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® SARS-CoV-2 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 the AltoStar® 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. Analyse 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.

8.2 Starting the AltoStar® AM16

1. Turn on the AltoStar® AM16 with the front left green switch and start the computer by pressing the power button.

2. Wait until Windows has booted.

3. Start the AltoStar® Connect software using the **a*** icon on the Windows desktop, the Windows task bar or in the Windows start menu.

The Start Screen of the AltoStar® Connect software is displayed (see Figure 1) showing three buttons representing the AltoStar® Workflow steps to be performed on the AltoStar® AM16:

- **Program Run:** Sample data are entered and assays are assigned to the samples. The programmed samples are then assigned to an AltoStar® Run (see chapter 8.5 Creating an AltoStar® Run), which includes one Purification Run and one or more PCR Setup and PCR runs. Several AltoStar® Runs can be preprogrammed in advance.

- **Start Purification:** A programmed Purification Run is selected and started as described in chapter 8.6 Starting a Purification Run.

- **Start PCR Setup:** A programmed PCR Setup Run is selected and started as described in chapter 8.11 Starting a PCR Setup Run.
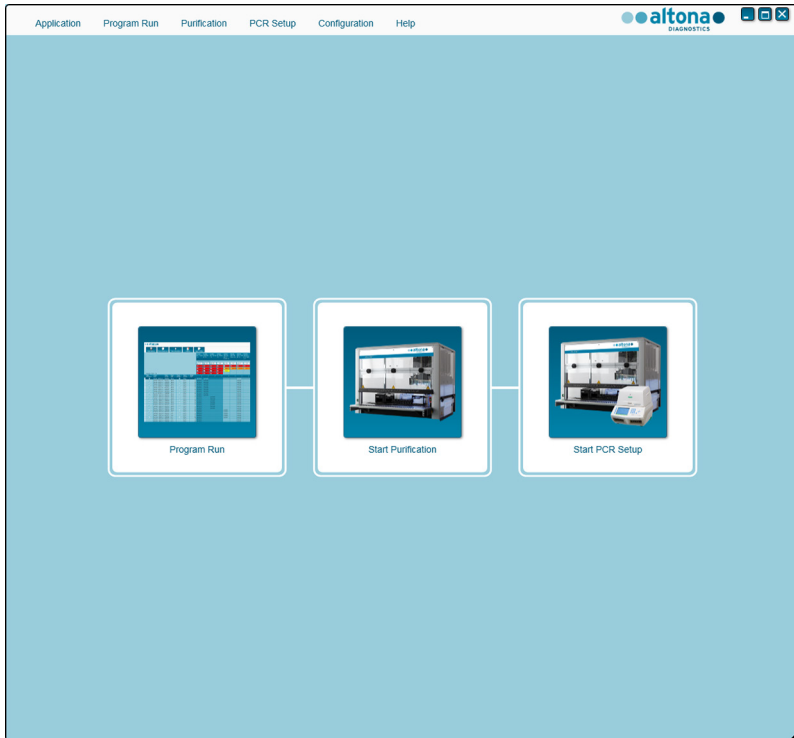
Figure   1: Start Screen of the AltoStar® Connect software

8.3 Performing Maintenance

1. Access the Maintenance Screen by clicking **Application → Instrument Maintenance** in the menu bar (see Figure 1).

A valid status of the Daily Maintenance and Weekly Maintenance is depicted by a green check mark in the column **Status** (see Figure 2). If a red crossed circle is displayed, the respective maintenance procedure has to be performed.
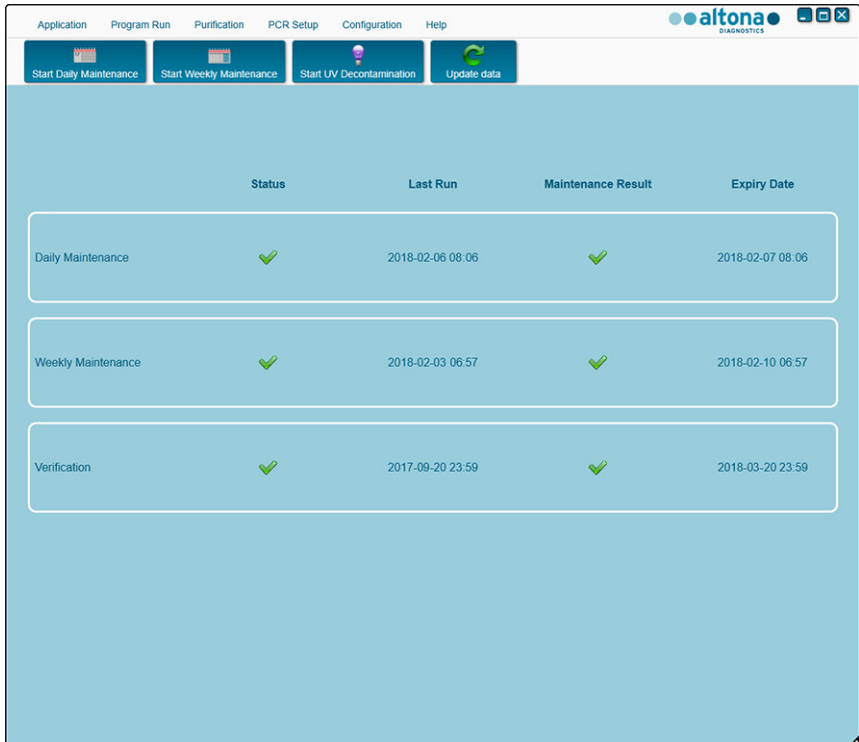
If the Daily or Weekly Maintenance has to be performed:

1. Click the corresponding button in the tool bar.

2. Follow the on screen instructions to complete the maintenance procedure. Refer to the AltoStar® Automation System AM16 Operator's Manual IVD and the AltoStar® Connect Software Manual IVD for detailed information.

The maintenance routines verify the correct functionality of the instrument and will prompt all necessary user actions including cleaning of the instrument.

NOTE
i   **Verification** refers to the semi-annual maintenance procedure that is performed by Hamilton trained field service engineers. The **Verification** row must show a green check mark in the column **Status** as well. Otherwise the instrument will not process any samples or reagents.

Figure 2: Maintenance Screen with valid maintenance status

8.4 Programming an AltoStar® Run

Input of sample data and assay assignments can be done manually (see chapter 8.4.1 Manual Programming) or by import from a connected Laboratory Information Management System (LIMS). If no manual programming is necessary, continue with chapter 8.4.2 Importing from LIMS.

8.4.1 Manual Programming

1. Click **Program Run** → **Program Run** (AltoStar® Purification) in the menu bar. Alternatively, go back to the Start Screen of the AltoStar® Connect software and select the **Program Run** button.

The Programming Screen is displayed (see Figure 3) showing the sample table at the bottom of the screen with columns for:

- Sample properties: **Sample Name** (optional), **Sample Barcode**, **Sample Type** and **Predilution**

- Sample settings: **Process Sample, Sample Priority**

- Sample information: required **Sample Volume** for the Purification Run (dead volume not factored in), **Eluate left** (determined by assay assignment)

- Assay assignment to the samples: **Programming**

NOTE

ℹ️ The sample settings **Process Sample** and **Sample Priority** are selected manually while the sample information **Sample Volume** and **Eluate left** are set automatically when assigning PCR assays to the samples.
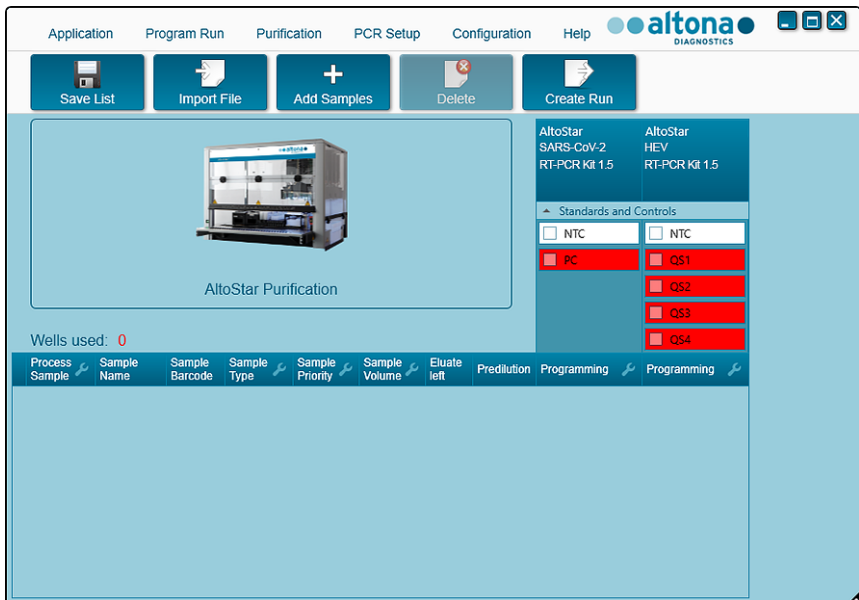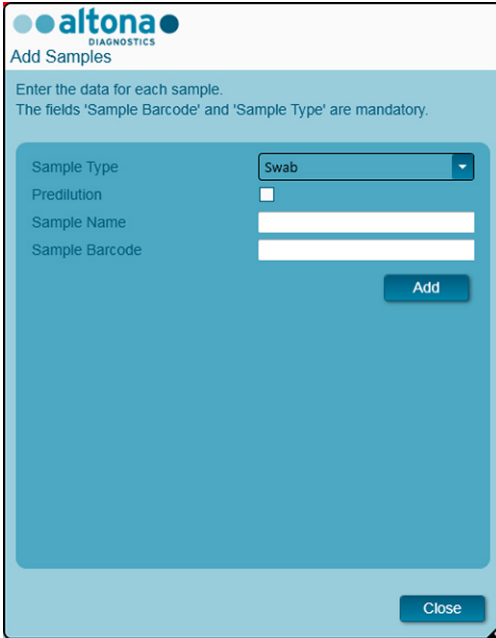

Figure   3: Programming Screen

2. Click the **Add Samples** button to manually add samples to the sample table. The **Add Samples** dialog will appear (see Figure 4).

Figure 4: Add Samples dialog

3. Select the sample type Swab in the **Sample Type** field.

> **NOTE**
>
> ℹ If the wrong sample type is selected in the **Sample Type** field the sample may not be processed.

4. *Optional*: Enter a sample name in the **Sample Name** field.

5. Enter a barcode via the handheld barcode scanner in the **Sample Barcode** field. A unique barcode for each sample tube is required.

6. Check for each sample if the required sample volume of 500 µl plus dead volume of the sample tube used is available.

NOTE

ℹ Insufficient sample volume (e.g. due to lack of the required dead volume of the sample tube) will lead to the exclusion of the sample in the Purification Run.

7. Tick the **Predilution** checkbox if the sample needs to be prediluted during the sample preparation procedure (see chapter 8.6.1 Sample Preparation) to provide the required sample volume.

- The **Sample Volume** field and **Added Diluent** field will appear (see Figure 5), each with 500 µl as preset volumes.

- Change the preset volumes of 500 µl in the **Sample Volume** field and **Added Diluent** field to match the volumes that will be used during the sample preparation.

Figure 5: Add Samples dialog: Ticked Predilution checkbox

> **NOTE**
>
> **i** The predilution will be included in the *Concentration Factor*, which is reported in the PCR results to calculate the target concentration in the original sample from the PCR results (see chapter 10.21.6.1 Manual Interpretation of Results). The calculation is either done by the user or by the LIMS during import of the PCR results.

> **NOTE**
>
> **i** The predilution property of a sample can be edited after closing the **Add Samples** dialog by ticking the checkbox in the column **Predilution** of the sample table.

8. Click the **Add** button to add the sample to the sample table.

9. Repeat the steps above until all samples are added to the sample table.

10. When all samples are added, click the **Close** button to close the **Add Samples** dialog. The added samples are displayed in the sample table of the Programming Screen (see Figure 6).
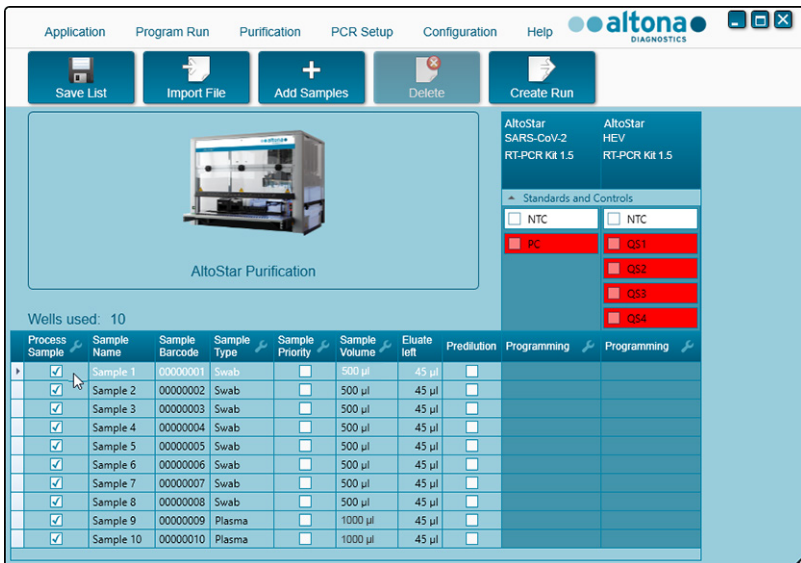

Figure 6: Programming Screen with added samples

> **NOTE**
>
> **i** The sample list can be sorted by individual columns by clicking the column header. Multiple samples can be selected by holding down the **Shift-Key** or **Ctrl-Key** while clicking on sample lines. The selected samples can be modified collectively by clicking the wrench symbol in the appropriate column header. Samples can be removed from the list by selecting them and clicking the **Delete** button in the tool bar.

11. Assign the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 assay to specific samples by clicking in the cell which is in the row of the respective sample and in the column of the **AltoStar® SARS-CoV-2 RT-PCR Kit 1.5** (see Figure 7).

12. Select **qualitative** in the appearing menu.
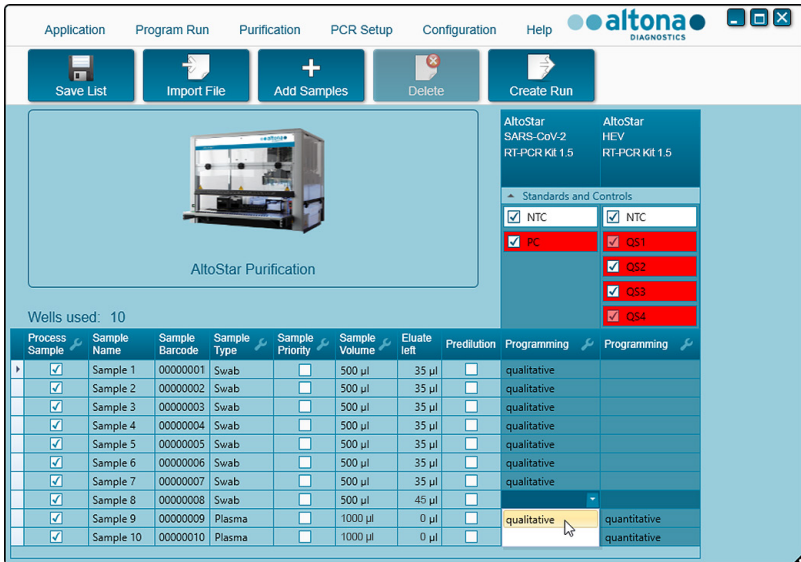
Figure 7: Programming Screen: PCR Assay Assignment

The correct set of **Controls** is automatically selected. Additionally, the required sample volume for the Purification Run (dead volume not factored in) and the eluate volume that remains available for assignment to other assays are automatically adjusted in the sample list columns **Sample Volume** and **Eluate left**, respectively.

> **NOTE**
>
> ℹ
>
> AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 settings:
>
> - PC and NTC are selected.
>
> - The required sample volume is 500 µl plus the dead volume for the respective sample tube (see chapter 7.3 Sample Volume).
>
> - The required eluate volume for the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 is 10 µl.

> **NOTE**
>
> ℹ
>
> If it is not possible to select a PCR assay for a sample, check in the **Eluate left** column of the sample table, whether the eluate volume required for this assay is still available.

8.4.2 Importing from LIMS

Both the sample properties as well as the assay assignment can be imported from the LIMS. To do so, click the **Import File** button in the tool bar. In the dialog that opens, select the Import File (.psv) that contains the required information.

For information regarding the LIMS integration, contact altona Diagnostics Technical Support (see chapter 10 Technical Assistance).

8.5 Creating an AltoStar® Run

For processing the samples in the sample table must be assigned to an AltoStar® Run which includes the Purification Run as well as one or more PCR Setup Runs and PCR Runs for a given sample.

1. Tick the **Sample Priority** checkbox for samples that should be sorted to the same PCR Plate for fastest processing.

   - Initially, all samples are ticked in the column **Process Sample** indicating that the respective samples are to be included in the AltoStar® Run generated next.

   - Above the sample table in the Programming Screen (see Figure 6), **Wells used** is displayed (showing the number of the AltoStar® Processing Plate wells needed for processing of the samples currently ticked in the column **Process Sample**).

   - Up to 96 wells can be used in one Purification Run.

NOTE

The AltoStar® Processing Plate is a consumable for Purification Runs and contains 96 wells that can be used for processing of samples. Samples with a processing volume of 1000 µl need two wells of the AltoStar® Processing Plate. Thus, the maximum number of samples that can be processed in one Purification Run varies and depends on the number of samples with a processing volume of 1000 µl.

   - If the number of 96 wells is exceeded, the AltoStar® Run cannot be created and **Wells used** is displayed in red.

2. In this case, deselect samples in the column **Process Sample** until **Wells used** displays 96 or less. The remaining samples still ticked in the column **Process Sample** will be assigned to the next AltoStar® Run.

3. Click the **Create Run** button in the tool bar of the Programming Screen. The **Save Run Definition** dialog is displayed (see Figure 8).

NOTE

No further modifications to samples are possible after clicking the **Create Run** button. If changes to a created AltoStar® Run are necessary, the created AltoStar® Run has to be deleted and manual programming or the import from LIMS has to be repeated.

---

4. Enter a unique **Run Name** and optionally a **Description** for identification of the AltoStar® Run later on.

5. Click the **Ok** button to save the AltoStar® Run.
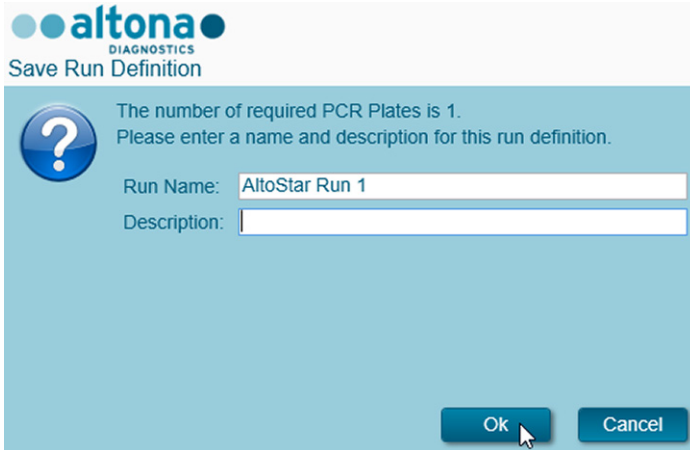

Figure   8: Save Run Definition dialog

Samples that have been assigned to an AltoStar® Run are removed from the sample table of the Programming Screen. To create further AltoStar® Runs for the remaining samples in the sample table:

6. Select up to 96 of the remaining samples in the column **Process Sample**.

7. Click the **Create Run** button and repeat the steps 4 and 5.

8.6 Starting a Purification Run

1. Select **Purification → Start Purification** in the menu bar. Alternatively, go back to the Start Screen of the AltoStar® Connect software and select the **Start Purification** button.

    •   The Start Purification Run Screen is displayed (see Figure 9). Each programmed AltoStar® Run includes one Purification Run.

    •   The pending Purification Runs are displayed in the **Programmed Purification Runs** table on the left side of the screen.
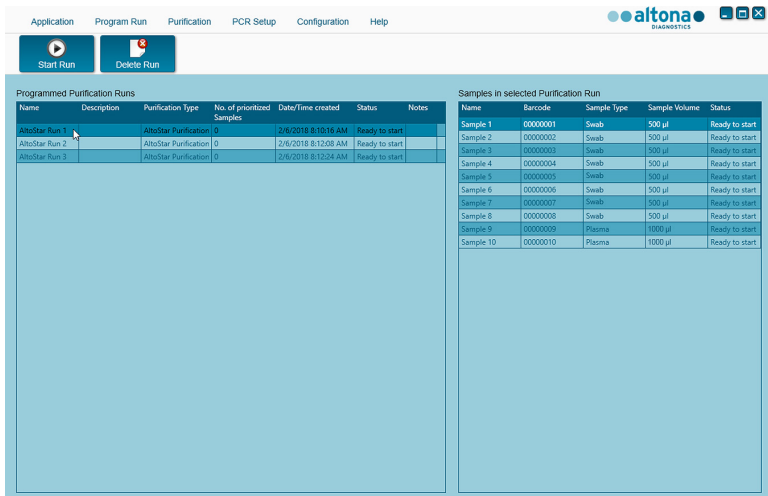
Figure   9: Start Purification Run Screen

2. Select the Purification Run to be started in the **Programmed Purification Runs** table. The samples included in the selected Purification Run are displayed in the table on the right side of the screen (**Samples in selected Purification Run**).

Before clicking the **Start Run** button in the tool bar, prepare the samples of the selected Purification Run and the reagents as described in chapters 8.6.1 Sample Preparation and 8.6.2 Preparing Reagents for a Purification Run.

8.6.1 Sample Preparation

For correct results the specifications regarding sample type, sample collection, sample volume, sample tube and sample barcode (see chapter 6 Sample Preparation) as well as with respect to sample preparation have to be followed carefully.

1. Prepare all samples that shall be used in the next Purification Run. The samples required for the selected Purification Run are listed in the table (**Samples in selected Purification Run**) on the right side of the Start Purification Run screen.

2. Provide at least 500 µl sample volume plus the required dead volume in a suitable sample tube.

NOTE

The samples must be free of solids and high-viscosity constituents. Solids and high-viscosity constituents will interfere with the sample transfer on the AltoStar® AM16 and the samples will not be processed.

NOTE

The sample volume is not checked by the system prior to processing. Samples with insufficient volume will not be processed and error flagged during the sample transfer step.

NOTE

If the samples must be prediluted: Predilution diluent, which is not compatible with this application may affect nucleic acid stability, sample transfer and purification performance.

8.6.2 Preparing Reagents for a Purification Run

1. Ensure to prepare sufficient amounts of non-expired reagents which all have to have the same *Loading Number*.

The *Loading Number* consists of the last four lot number digits of the Lysis Buffer and Wash Buffer containers and the Magnetic Beads, Enhancer and Elution Buffer tubes.

Figure 10: Loading Number

> **NOTE**
>
> **i**
>
> Before processing starts the AltoStar® AM16 automatically verifies
>
> 1) that sufficient reagent volume of the AltoStar® Purification Kit 1.5 components and of the AltoStar® Internal Control 1.5 is present,
>
> 2) that the *Loading Numbers* of the loaded AltoStar® Purification Kit 1.5 components are congruent.

2. Visibly inspect the Lysis Buffer for precipitates. In case precipitates are visible, heat it to below 50 °C. Intermittently pivot the container gently without wetting the seal until precipitates are completely dissolved. Slight color changes may occur to the Lysis Buffer. These slight changes in color do not indicate a change in the quality of the buffer.

3. Vortex the Magnetic Bead tubes for 5 seconds. Avoid wetting the lid. Do not centrifuge the Magnetic Beads.

4. Thaw the required number of IC tubes (AltoStar® Internal Control 1.5) completely and vortex for 5 seconds.

8.6.3 Instrument Loading for a Purification Run
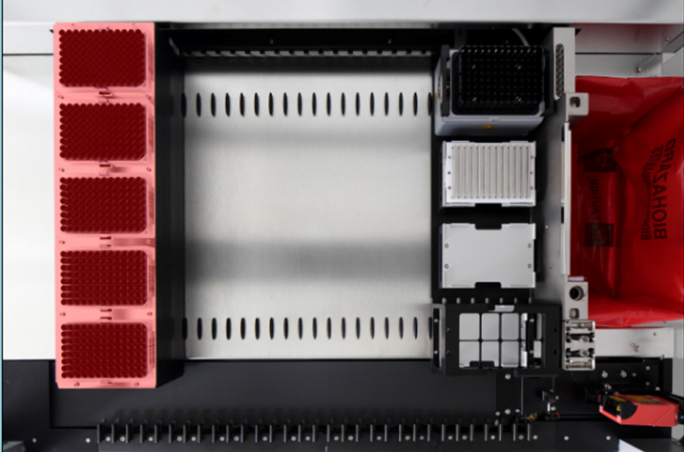
1. Click the **Start Run** button in the tool bar of the Start Purification Run screen to display the **Loading** dialog (see Figure 11).

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

Figure 11: Loading dialog

> **NOTE**
>
> ℹ 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:
>
> 1) Highlighted in red in the visual representation of the instrument deck.
>
> 2) On the AltoStar® AM16 by flashing Loading Lights above the tracks where the selected carrier must be placed.

2. Load the material, prepared reagents and prepared samples onto the suitable carriers as follows:

Track	Carrier Description	Material to be loaded
1 - 6	One Tip Carrier	5 x 1000 µl Tip Racks

- Exchange only **completely empty** 1000 µl Tip Racks for **completely full** 1000 µl Tip Racks on the Tip Carrier.

> **NOTE**
>
> ℹ 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.
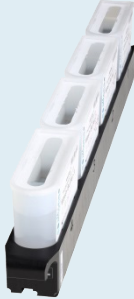
Track	Carrier Description	Material to be loaded
7 - 12	One Tip and Plate Carrier	3 x 300 µl Tip Racks 1 x Eluate Plate

- Exchange only **completely empty** 300 µl Tip Racks for **completely full** 300 µl Tip Racks on the Tip and Plate Carrier.

- Place the Eluate Plate with well A1 to the left of the black plate position. The plate position at the front is not used during Purification Runs.

NOTE

ℹ️ 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.

Track	Carrier Description	Material to be loaded
13 - 16	One or two Container Carriers	up to 8 containers of: Lysis Buffer Wash Buffer 1 Wash Buffer 2 Wash Buffer 3

- Load one or two Container Carriers with up to eight containers of Lysis Buffer, Wash Buffer 1, Wash Buffer 2 and Wash Buffer 3.

- Gently push the containers all the way to the bottom of the carrier.

- Remove and dispose of all sealing foils from the containers.

NOTE

i Starting a Purification Run with the sealing foils still on the containers may cause the run to abort during processing.

NOTE

i The position of the individual containers on the respective carriers is arbitrary.

Track	Carrier Description	Material to be loaded
17	 One Tube Carrier 24	up to 24 tubes of: IC (Internal Control) Magnetic Beads Enhancer Elution Buffer

- Load a Tube Carrier 24 with up to 24 tubes of IC, Magnetic Beads, Enhancer and Elution Buffer.

- 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 windows.

- Remove all lids from the tubes and store them for reuse.

- Store the lids for reuse in a clean space.

NOTE

ℹ Reuse of lids for any other tube than the original one may lead to cross-contamination.

NOTE

ℹ The position of the individual tubes on the carrier is arbitrary.

NOTE

ℹ Starting a Purification Run with lids still on the tubes may cause the run to abort during processing.

Track	Carrier Description		Material to be loaded
18 - 23	One to six Tube Carrier 32 for sample tubes of 11 - 14 mm diameter	One to six Tube Carrier 24 for sample tubes of 14.5 - 18 mm diameter	Prepared samples for the Purification Run to be started

- Load the prepared samples for the Purification Run on up to six sample carriers. Two carrier types can be used in parallel in the same run:

    ◦ For sample tubes of 11 - 14 mm outer diameter use the Tube Carrier 32.

    ◦ For sample tubes of 14.5 - 18 mm outer diameter use the Tube Carrier 24.

- 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 windows.

NOTE

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

NOTE

ℹ Starting a Purification Run with lids still on the tubes may cause the run to abort during processing.

Track	Carrier Description	Material to be loaded
24 - 30	 Heater Shaker Carrier This carrier is not removable. The items are placed by hand onto the carrier in the instrument.	1 x AltoStar® Processing Plate 1 x Tip Park Plate 1 x Tip Park Rack

- Place an unused Tip Park Plate at the bottom of the front position and an unused Tip Park Rack at the top of the front position and ensure both items are latched into their respective position.

- Place an unused AltoStar® Processing Plate at the second position from the front and ensure it is latched into position.

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

ℹ 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

ℹ By clicking **Cancel** the Purification Run will be cancelled, but it can be started again (see chapter 10.6 Starting a Purification Run).

The **Tip Park Plate** dialog is displayed (see Figure 12).

Figure 12: Tip Park Plate dialog

7. Scan the Tip Park Plate barcode in duplicate with the handheld barcode scanner to ensure that the plate has not been used in prior runs.

8. Click **Ok** to confirm the input.

The AltoStar® AM16 draws the carriers into the instrument and performs barcode and reagent volume verifications.

> **NOTE**
>
> ℹ
>
> The AltoStar® AM16 automatically verifies:
>
> 1) Correct type and localization of the loaded carriers
>
> 2) Correct identity and position of the items loaded on the carriers
>
> 3) Lot congruence of the AltoStar® Purification Kit 1.5 components (Lysis Buffer, Wash Buffers, Magnetic Beads, Enhancer and Elution Buffer)
>
> 4) Non-expiry of all loaded reagents
>
> 5) Presence of sufficient reagent volumes
>
> 6) Singularity of sample barcodes
>
> 7) Correct positioning of the items loaded manually on the Heater Shaker Carrier
>
> 8) 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 AltoStar® Connect Software Manual IVD (Hamilton, chapter 4 Troubleshooting and Error Messages).

> **NOTE**
>
> ℹ
>
> Altering of positions of any loaded item after the carrier has been drawn into the instrument results in abort of the Purification Run and damage to the instrument.

When all checks have passed the **Loading complete** dialog is displayed (see Figure 13).

Figure 13: Loading complete dialog

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

NOTE

By clicking **Cancel** the Purification Run will be cancelled, but it can be started again (see chapter 10.6 Starting a Purification Run).

The Purification Run is started and will be conducted without user intervention.

8.7 During the Purification Run

No further user interaction is required until the Purification Run has finished. The **Processing Status Screen** is displayed (see Figure 14) showing the status of the Purification Run and the estimated time remaining.
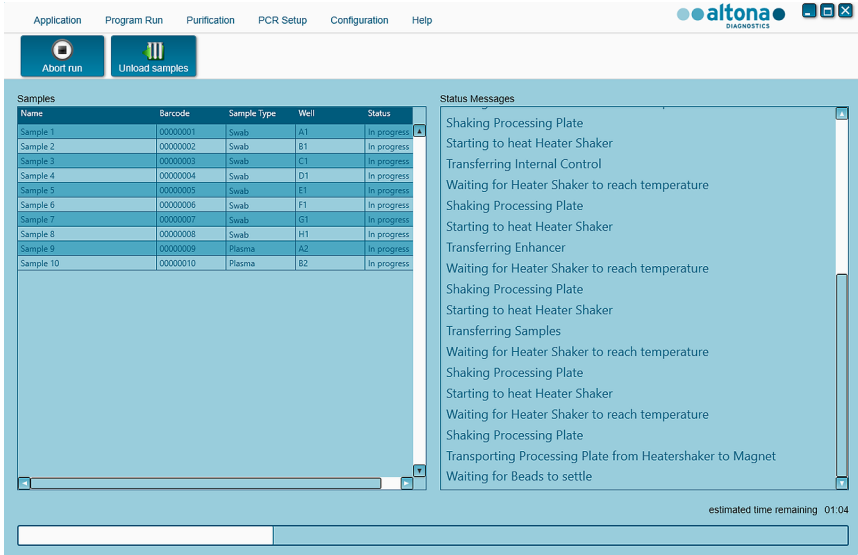
Figure   14: Processing Status Screen

NOTE

ℹ️ Pushing or pulling carriers or the door of the AltoStar® AM16 during a Purification Run may abort the run.

NOTE

ℹ️ Aborting the Purification Run after the **Loading complete** dialog is confirmed will void the AltoStar® Run, preventing a restart. To repeat aborted runs see the AltoStar® Connect Software Manual IVD (Hamilton, chapter 3.8.14 Purification Results).

NOTE

ℹ️ After the sample transfer into the AltoStar® Processing Plate has finished, the sample carrier(s) can be unloaded at any time. The **Unload samples** button in the tool bar will be active and can be clicked. The sample carrier(s) will be unloaded from the deck and the sample tubes can be removed. The Purification Run will not be interrupted.

> **NOTE**
>
> Required components of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 for the subsequent PCR Setup Run can be previewed to allow for preparation of these components during the preceding Purification Run:
>
> 1. Click **PCR Setup → Start PCR Setup** in the menu bar to access the Start PCR Setup Run Screen.
>
> 2. Refer to the tables **Controls in selected PCR Setup Run** and **Required master tubes for the selected PCR Setup Run** for information on the required components.
>
> 3. Return to the ongoing Purification Run by clicking **Purification → Current Purification** in the menu bar.

8.8 End of the Purification Run

At the end of the Purification Run the **Run finished** dialog is displayed (see Figure 15).
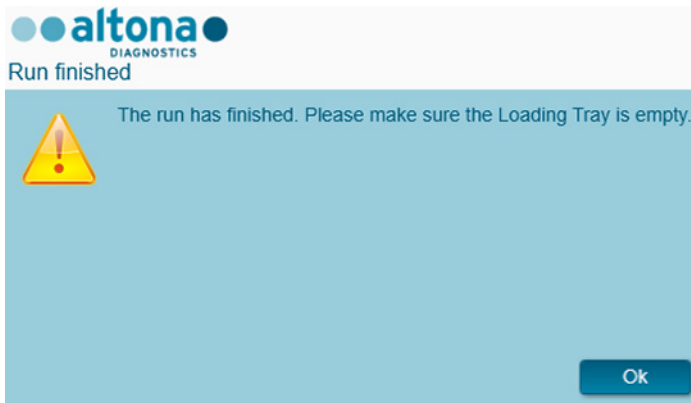
Figure   15: Run finished dialog

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 (see Figure 16).

3. Follow the instructions of the **Maintenance** dialog.
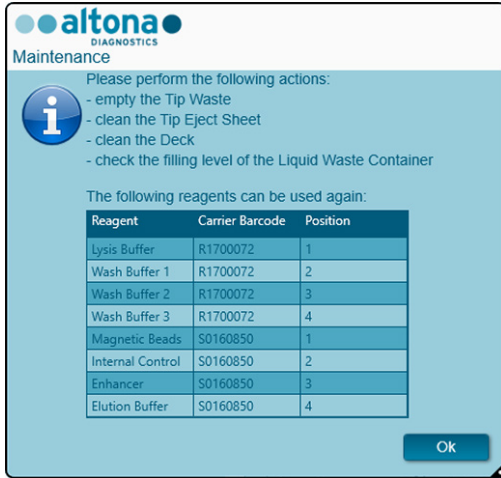
Figure   16: Maintenance dialog

The table of the dialog displays components of the AltoStar® Purification Kit 1.5 and the IC (AltoStar® Internal Control 1.5) with sufficient volume to be used again in subsequent Purification Runs.

1. If a PCR Setup Run using the currently loaded Eluate Plate is to be started directly after the Purification Run, the Eluate Plate can remain on the carrier position at room temperature (max. 30 °C) up to 6 hours. If the PCR Setup Run is **not** started directly after the Purification Run, seal and store the Eluate Plate as described in chapter 8.10.1 Sealing of the Eluate Plate.

2. Close tubes with the appropriate tube caps. Avoid interchanging the tube caps when closing the reagents after use.

3. Close containers with unused AltoStar® Container Re-Sealing Foils.

4. Store reagents for reuse as described in the chapters 4 Storage and Handling in the Instructions for Use of the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5, respectively.

5. Dispose of the components of the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 **not** listed in the table.

Dispose of the samples and used materials (see chapter 9 Disposal).

6. Confirm the **Maintenance** dialog by clicking **Ok**.

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

NOTE
i   Liquid waste and any liquids containing Lysis Buffer or Wash Buffer 1 contain guanidine thiocyanate, which can form toxic, highly reactive and volatile compounds when combined with bleach or strong acids.

NOTE
i   The instructions for the daily maintenance procedure for the disposal of liquid waste and used materials can be found in the AltoStar® AM16 Operator´s Manual IVD (Hamilton, chapter 3.5 Maintenance).

8.9 Purification Run Results

The Purification Run results are saved in the AltoStar® Connect software.

1. Click **Purification** → **Purification Results** in the menu bar to access the Results Screen (see Figure 17).

Figure 17: Results Screen

The Results Screen displays a table with all samples used in the latest Purification Run and a column **Status** at the right showing if the Purification Run for a given sample was conducted completely (see Table 5).

Table 5: Purification Run Results

Status	Purification Run Result
Processed	• The sample was successfully processed in the Purification Run. • The respective eluate is ready for use in a PCR Setup Run.
Error	• The sample was not processed successfully. • No eluate of this sample is available. • The sample will be automatically omitted from following PCR Setup Runs.

2. To view the results of prior Purification Runs, click the **Load** button in the menu bar, select the desired Purification Run from the list in the opening **Load Results** dialog and click **Ok**.

Two Purification Run result files are automatically generated by the AltoStar® Connect software:

• A LIMS file (.xml) to pass detailed information about the Purification Run including results back to the LIMS.

• A report (.pdf) containing detailed information about the Purification Run including results for documentation purposes.

These files are saved to the location specified in the System Settings of the AltoStar® Connect software.

NOTE

ℹ Purification Run result files can be generated again by loading the respective Purification Run and clicking the **Create LIMS File** button to generate the LIMS file or the **Create Report** button to generate the report.

8.10 Sealing and Unsealing of the Eluate Plate

8.10.1 Sealing of the Eluate Plate

In case the eluates in the Eluate Plate are to be stored, the plate must be sealed with AltoStar® Eluate Plate Sealing Foil. It is recommended to use the AltoStar® Plate Sealer. The suitability of plate sealers other than the AltoStar® Plate Sealer has to be evaluated by the user.

NOTE

i Using unsuitable plate sealers or sealing parameters may damage the eluates as well as the Eluate Plate, the AltoStar® Eluate Plate Sealing Foil and the plate sealer.

If the AltoStar® Plate Sealer is used for sealing, proceed as follows:

1. Turn on the AltoStar® Plate Sealer and make sure that the plate adapter is not in the drawer.

2. Ensure that the settings of the AltoStar® Plate Sealer are as follows: 170 °C and 2 seconds.

3. Wait until the set temperature of 170 °C is reached. This may take several minutes.

4. Place the Eluate Plate on the plate adapter of the AltoStar® Plate Sealer.

5. Place one AltoStar® Eluate Plate Sealing Foil on the Eluate Plate. Align the cut corner of the sealing foil with the cut corner of the Eluate Plate. Make sure that all wells of the Eluate Plate are covered with foil. Please take special care that the well at the cut corner is covered properly.

NOTE

i Operating the AltoStar® 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 15 Technical Assistance).

> **NOTE**
>
> **i** If the AltoStar® Eluate Plate Sealing Foil or the frame is placed incorrectly, the foil may stick to the heating plate within the AltoStar® Plate Sealer during sealing. This will render the sealer nonfunctional. In this case let the AltoStar® Plate Sealer cool down to room temperature and contact altona Diagnostics Technical Support for assistance (see chapter 15 Technical Assistance).

6. Assemble the sealing frame on top to hold down the sealing foil.

7. Open the drawer via the **Operate** button.

8. Place the assembly consisting of the plate adapter, the Eluate Plate, the AltoStar® Eluate Plate Sealing Foil and the sealing frame into the AltoStar® Plate Sealer and press the **Operate** button.

9. The drawer closes automatically, seals for 2 seconds and reopens automatically.

10. Take the sealed Eluate Plate and the plate adapter out of the AltoStar® Plate Sealer and close the AltoStar® Plate Sealer by pressing the **Close** button.

8.10.2 Unsealing of the Eluate Plate

Remove the AltoStar® Eluate Plate Sealing Foil from the Eluate Plate as follows:

1. Briefly centrifuge the Eluate Plate in a plate centrifuge to remove any liquid from the inside of the sealing foil.

2. Press the Eluate Plate onto a table to avoid sudden plate movements during the removal of the sealing foil.

3. Start peeling in one corner and slowly and steadily pull the sealing foil towards the diagonally opposite corner until it is removed.

8.11 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 (see Figure 18).

The pending PCR Setup Runs are displayed in the **Programmed PCR Setup Runs** table on the left side of the screen.
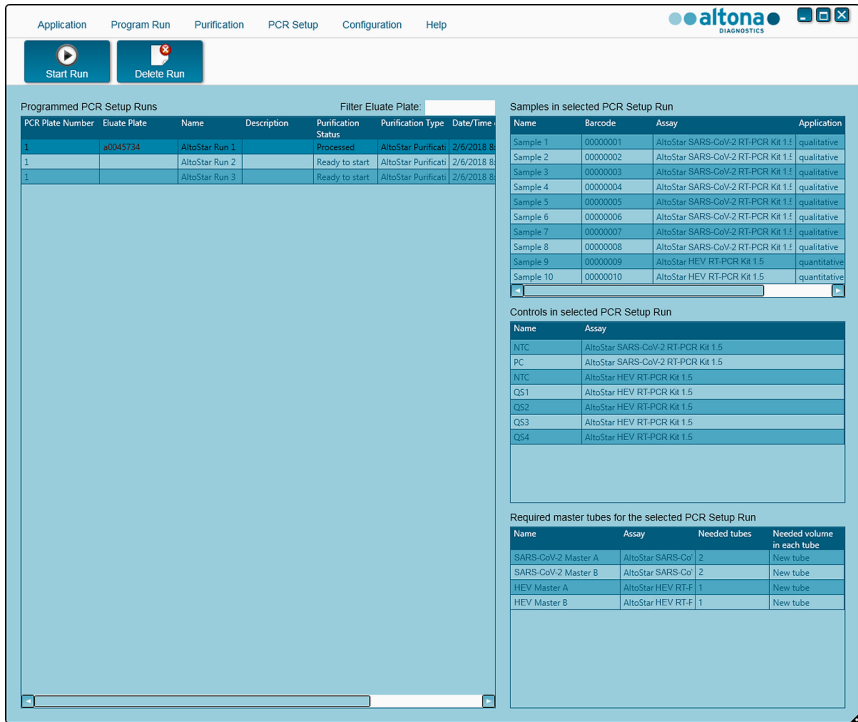
Figure 18: Start PCR Setup Run Screen

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 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**
>
> **i** 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 8.12 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 chapter 8.10.2 Unsealing of the Eluate Plate.

8.12 Preparing Reagents for a PCR Setup Run

1. Thaw the required 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.

> **NOTE**
>
> **i** A lack of centrifugation of the product components after thawing could lead to contamination of the components with reagent residues in the lids and as consequence to a compromised product performance.

8.12.1 Loading the AltoStar® AM16 for a PCR Setup Run

1. Click the **Start Run** button in the tool bar of the **Start PCR Setup Run Screen** to display the **Loading** dialog (see Figure 19).

Figure 19: 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:
1) Highlighted in red in the visual representation of the instrument deck.
2) 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 as follows:

Track	Carrier Description	Material to be loaded
1 - 6	 One Tip Carrier	5 x 1000 µl Tip Racks

- Exchange only **completely empty** 1000 µl Tip Racks for **completely full** 1000 µl Tip Racks on the Tip Carrier.

NOTE

ℹ️ 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.

Track	Carrier Description	Material to be loaded
7 - 12	One Tip and Plate Carrier	3 x 300 µl Tip Racks 1 x Eluate Plate 1 x PCR Plate

- Exchange only **completely empty** 300 µl Tip Racks for **completely full** 300 µl Tip Racks on the Tip and Plate Carrier.

- 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.

NOTE

ℹ️ 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.

Track	Carrier Description	Material to be loaded
13	One Tube Carrier 24	1 Pooling Tube per assay

- 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.

NOTE

ℹ The position of the individual tubes on the carrier is arbitrary.

Track	Carrier Description	Material to be loaded
14 - 17	 One to four Reagent Tube Carrier 32	Assay components

- 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

ℹ️ 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

ℹ️ By clicking **Cancel** the PCR Setup Run will be cancelled, but it can be started again (see chapter 10.11 Starting a PCR Setup Run).

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

NOTE

The AltoStar® AM16 automatically verifies:

1) Correct type and localization of the loaded carriers.

2) Correct identity and position of the items loaded on the carriers.

ℹ️ 3) Lot congruence of the components of the individual AltoStar® assay kits.

4) Non-expiry of all loaded AltoStar® assay components.

5) 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 AltoStar® Connect Software Manual IVD (Hamilton, chapter 4 Troubleshooting and Error Messages).

NOTE

ℹ️ 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 (see Figure 20).

Figure 20: Loading complete dialog

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

NOTE

ℹ️ By clicking **Cancel** the PCR Setup Run will be cancelled, but it can be started again (see chapter 10.11 Starting a PCR Setup Run).

The PCR Setup Run is started and will be conducted without user intervention.

8.13 During the PCR Setup Run

No further user interaction is required until the PCR Setup Run has finished. The Processing Status Screen is displayed (see Figure 21) showing the status of the PCR Setup Run and the estimated time remaining.
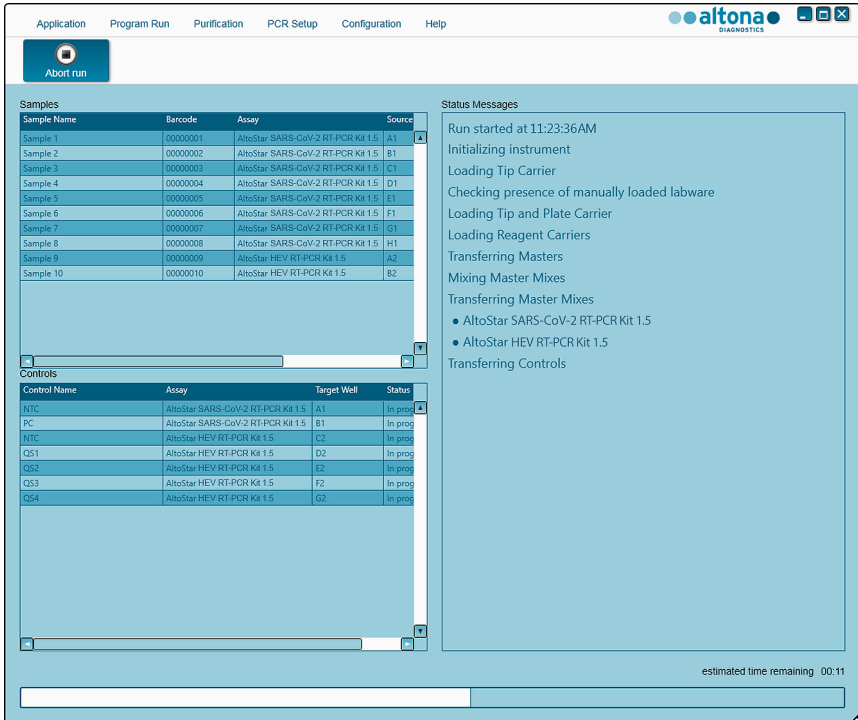
Figure   21: Processing Status Screen

NOTE

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

8.14 End of the PCR Setup Run

At the end of the PCR Setup Run the **Run finished** dialog is displayed (see Figure 22).
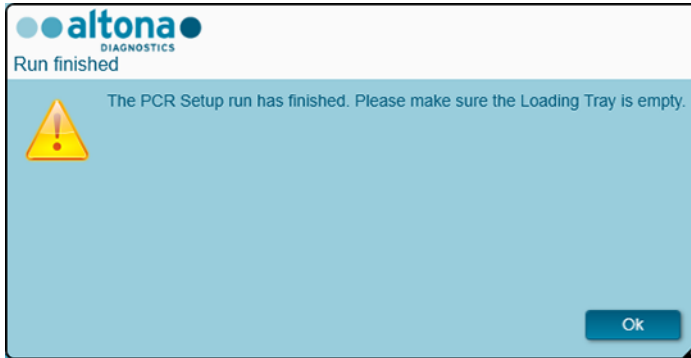
Figure   22: Run finished dialog

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 (see Figure 23).

3. Follow the instructions of the **Maintenance** dialog.
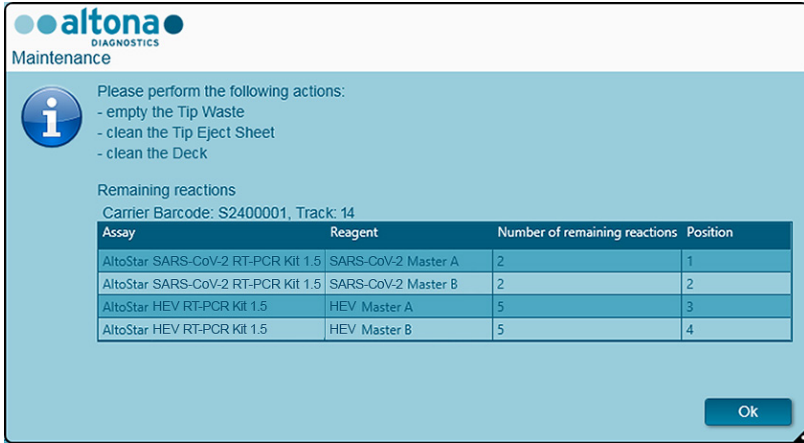
Figure 23: Maintenance dialog

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 as described in chapter 8.10.1 Sealing of the Eluate Plate.

NOTE

ℹ The eluates in the Eluate Plate are stable at room temperature (max. 30 °C) for a total of 6 hours after completion of the Purification Run.

5. Close reagent tubes with suitable unused tube caps.

NOTE

ℹ Do not reuse tube caps to avoid contamination of the reagents.

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**.

8.15 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 (see Figure 24).

Figure   24: 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**.

Three 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

ℹ 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.

8.16 Sealing of the PCR Plate

After completion of the PCR Setup Run, the PCR Plate must be sealed with AltoStar®
PCR Plate Sealing Foil. It is recommended to use the AltoStar® Plate Sealer. The
suitability of plate sealers other than the AltoStar® Plate Sealer has to be evaluated
by the user.

If the AltoStar® Plate Sealer is used for sealing, proceed as follows:

1. Switch on the AltoStar® Plate Sealer and make sure that the plate adapter is
   not in the drawer.
2. Ensure that the settings of the AltoStar® Plate Sealer are as follows: 170 °C
   and 2 seconds.
3. Wait until the set temperature of 170 °C is reached. This may take several
   minutes.
4. Place the PCR Plate on the plate adapter of the AltoStar® Plate Sealer.
5. Place one AltoStar® PCR Plate Sealing Foil on the PCR Plate. Align the cut
   corner of the sealing foil with the cut corner of the PCR Plate. Make sure that
   all wells of the Eluate Plate are covered with foil. Please take special care that
   the well at the cut corner is covered properly.

NOTE

ℹ️ Operating the AltoStar® 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 15
Technical Assistance).

NOTE

ℹ️ If the AltoStar® PCR Plate Sealing Foil or the frame is placed incorrectly,
the foil may stick to the heating plate within the AltoStar® Plate Sealer
during sealing. This will render the sealer nonfunctional. In this case let
the AltoStar® Plate Sealer cool down to room temperature and contact
altona Diagnostics Technical Support for assistance (see chapter 15
Technical Assistance).

---

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 AltoStar® PCR Plate Sealing Foil and the sealing frame into the AltoStar® Plate Sealer and press the **Operate** button.

9. The drawer closes automatically, seals for 2 seconds and reopens automatically.

10. Take the sealed PCR Plate and the plate adapter out of the AltoStar® Plate Sealer and close the AltoStar® Plate Sealer by pressing the **Close** button.

8.17 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 (see Figure 25).
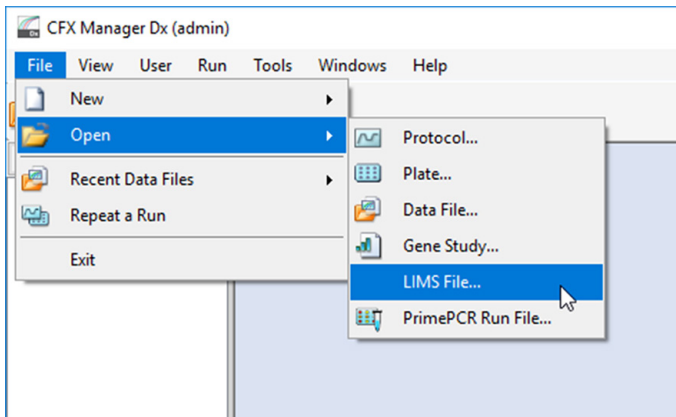
Figure 25: CFX Manager™ Dx software window

4. In the opening **Open LIMS File** dialog, make sure that the cursor is blinking in the **File name** field at the bottom (see Figure 26). If it is not, click into the **File name** field.
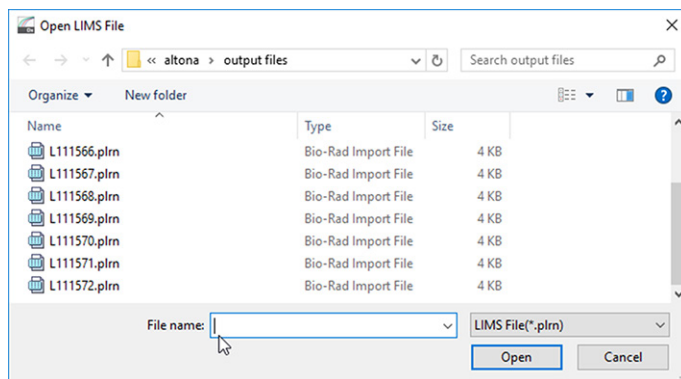
Figure 26: Open LIMS File dialog

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 (see Figure 27).

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.

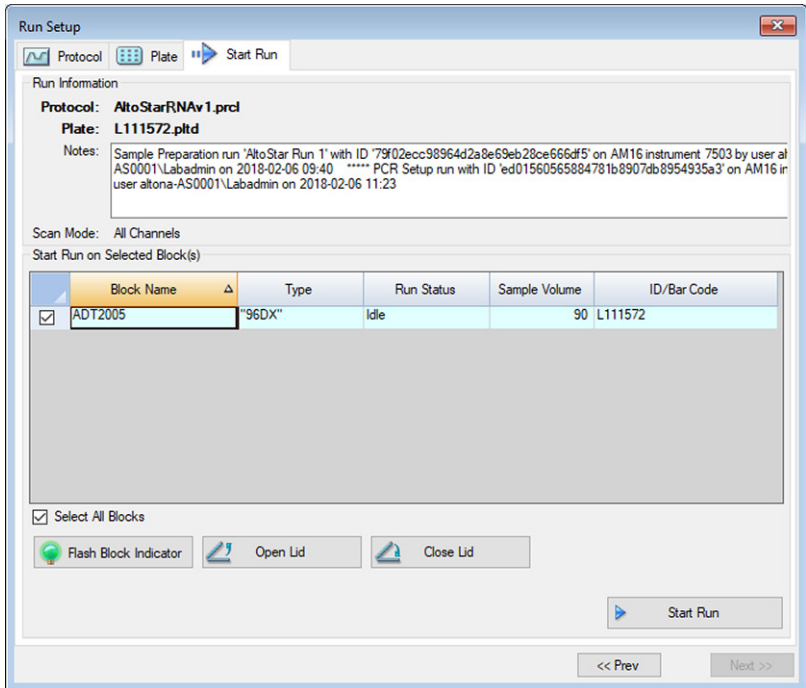6. Click **Open Lid** to open the lid of the CFX96™ DW Dx (see Figure 27).

Figure 27: Run Setup dialog

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 (see Figure 27).

10. Start the PCR Run by clicking the **Start Run** button in the **Run Setup** dialog (see Figure 27).

8.18 During the PCR Run

No user interaction is required until the PCR Run has finished. The **Run Details** dialog is displayed (see Figure 28) showing the status of the PCR Run and the estimated time remaining.
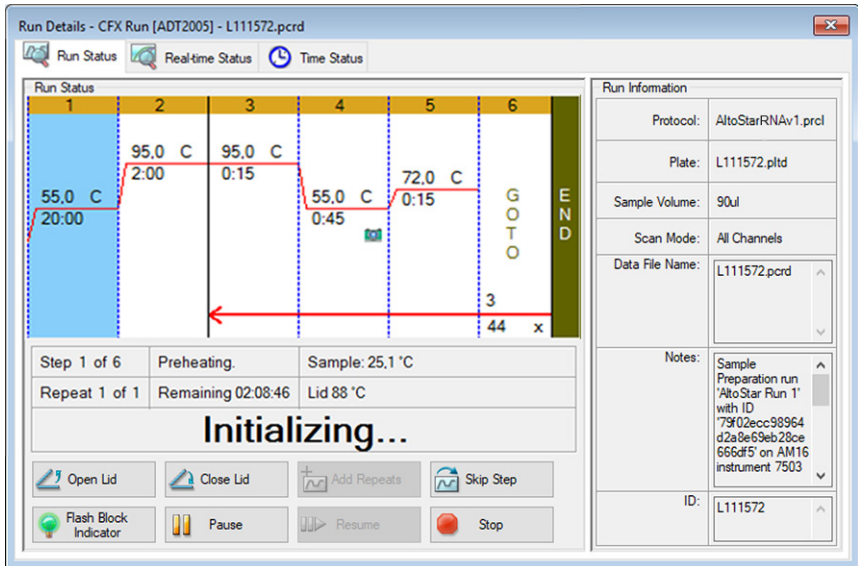
Figure   28: Run Details dialog

NOTE

ℹ️ 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 (see Figure 29).
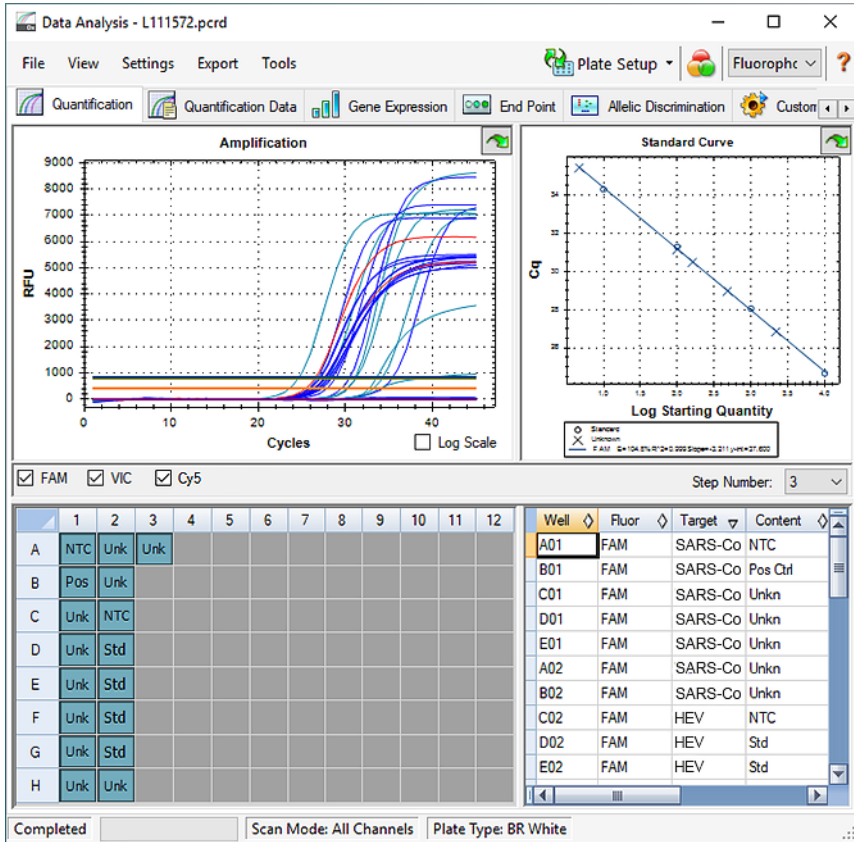
Figure 29: Data Analysis window

8.19 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 (see Figure 29) click the **Plate Setup** button in the tool bar and select **View/Edit Plate**. The **Plate Editor** dialog is displayed (see Figure 30).
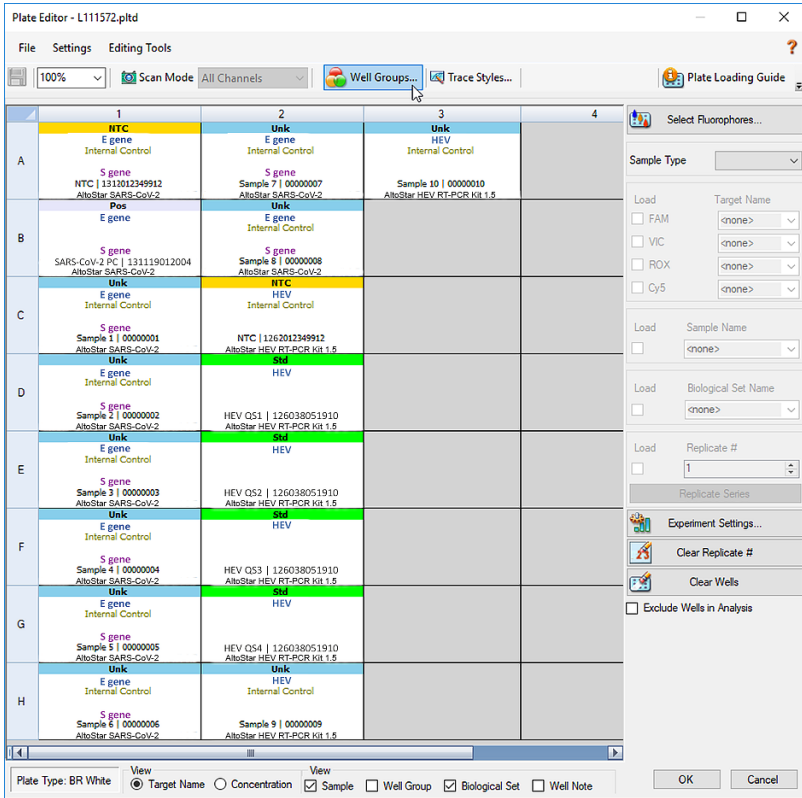
Figure 30: Plate Editor dialog

2. In the **Plate Editor** dialog click **Well Groups…** in the tool bar. The **Well Groups Manager** dialog is displayed (see Figure 31).

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 31). The wells belonging to an individual assay can be identified in the **Plate Editor** dialog by the entry in the **Biological Set** field.
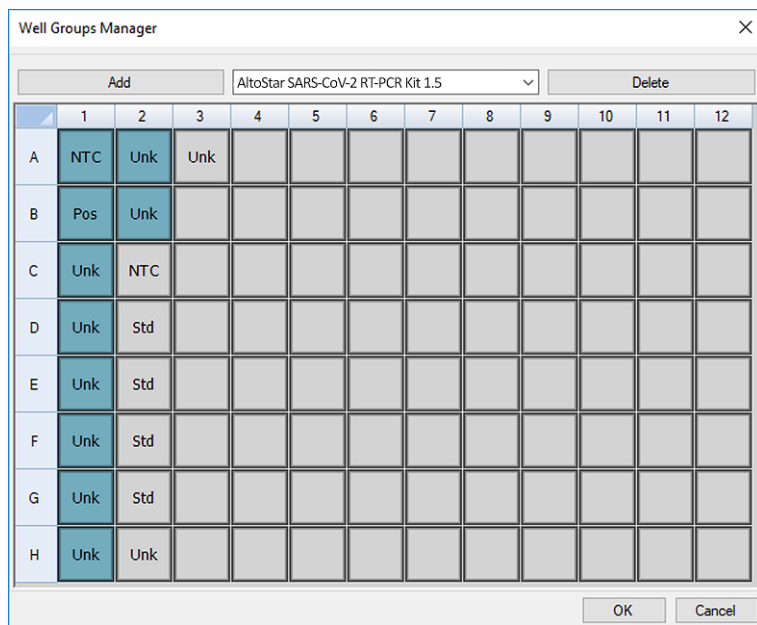
Figure   31: Well Groups Manager dialog

6. Repeat steps 3 to 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**.

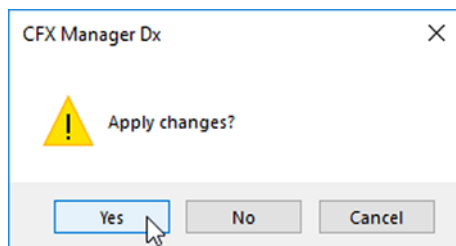9. Confirm to apply the changes by clicking **Yes** (see Figure 32).

Figure   32: Confirmation dialog

8.20 PCR Data Analysis

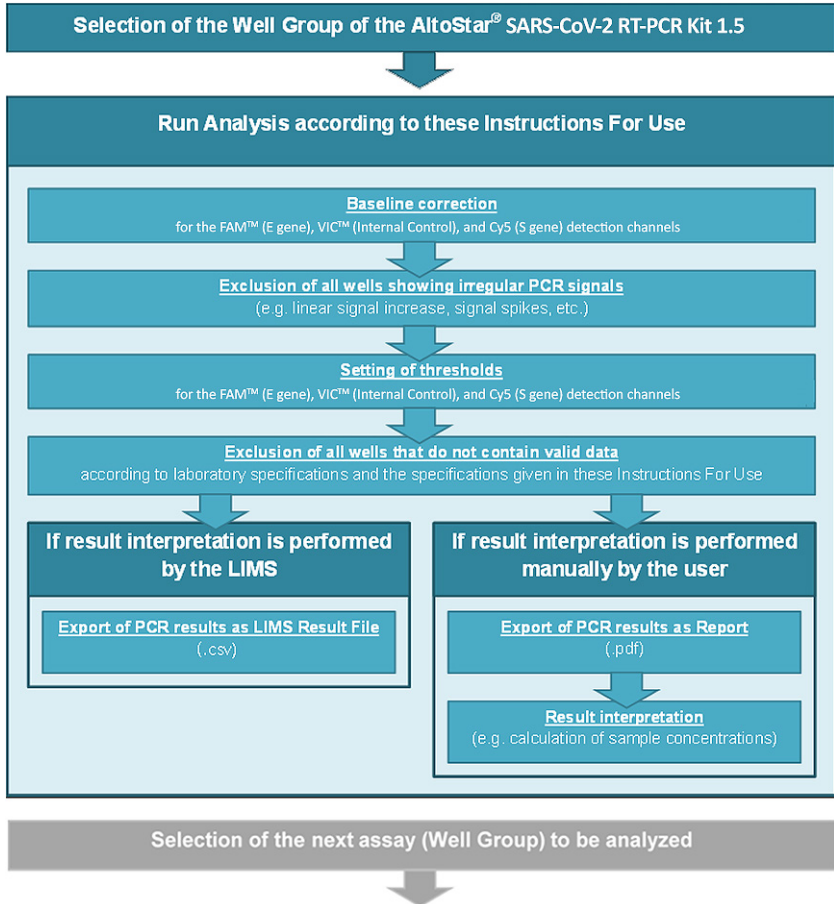The results of all assays (**Well Groups**) on the PCR Plate have to be analyzed in the sequence depicted in Figure 33.

Figure   33: PCR Data Analysis process

In the **Data Analysis** window (see Figure 29) ensure to select the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see Figure 34) of the toolbar. Do not use the *"All Wells"* **Well Group**.

Before analyzing the results ensure that the Well Group of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 contains all of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 wells and no wells of other assays.
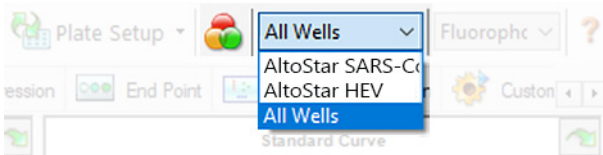

Figure   34: Well Group button and Well Group drop-down menu

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

8.20.1 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 (see Figure 29) ensure to select the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see Figure 34) of the toolbar.

2. At the left side of the **Data Analysis** window tick only the **FAM** checkbox for the E gene detection channel (see Figure 29).

3. In the menu bar of the **Data Analysis** window click **Settings → Baseline Threshold…** to open the **Baseline Threshold** dialog (see Figure 34).

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 to 9 (see Figure 35).
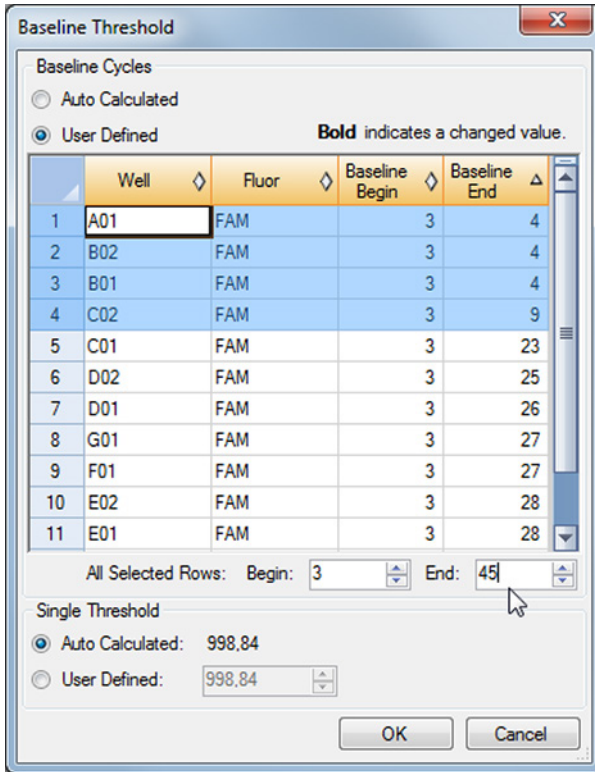

Figure 35: Baseline Threshold dialog

6. Set the value in the **End**: field to 45 for the selected lines (see Figure 35).

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 Internal Control target detection channel.

9. Repeat steps 3 to 7 for the VIC™ (Internal Control) and Cy5 (S gene) detection channels.

8.20.2 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 (see Figure 29) ensure to select the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to The **Well Group** button (see Figure 34) of the toolbar.

2. Identify wells with irregular PCR signals (linear signal increase, signal spikes, etc.) in any of the FAM™ (E gene), the Cy5 (S gene) and the VIC™ (Internal Control) detection channels (see Figure 36).
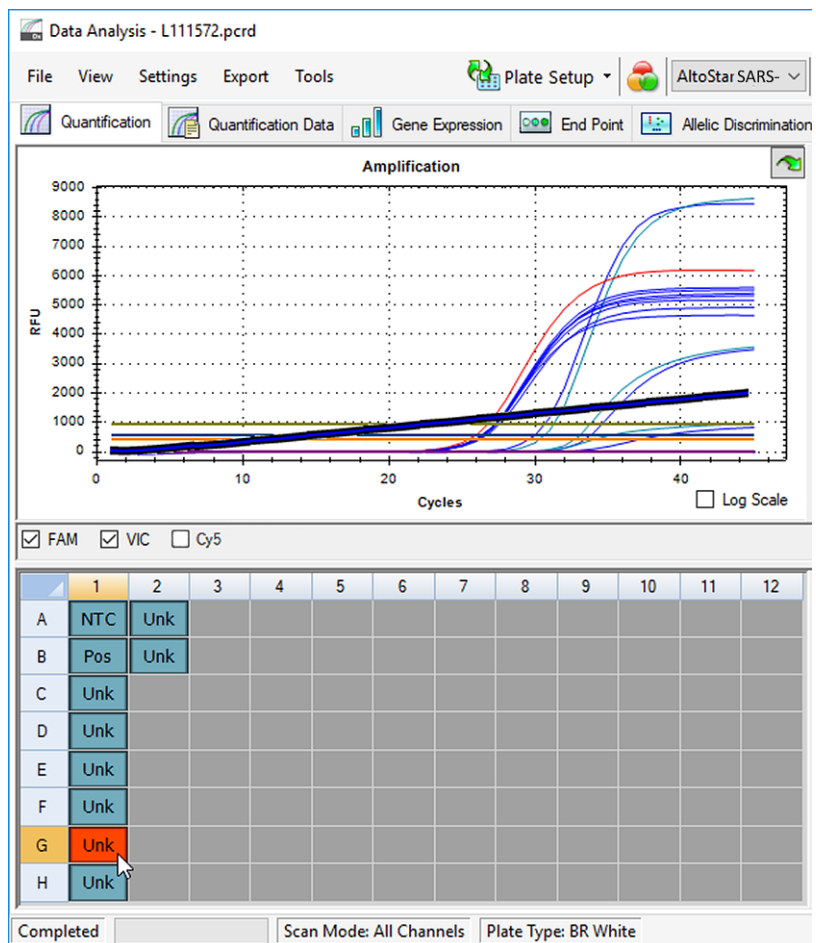
Figure 36: Data Analysis window: Irregular PCR signal

3. Click each affected well with the right mouse button and select **Well**... → **Exclude from Analysis** (see Figure 37).
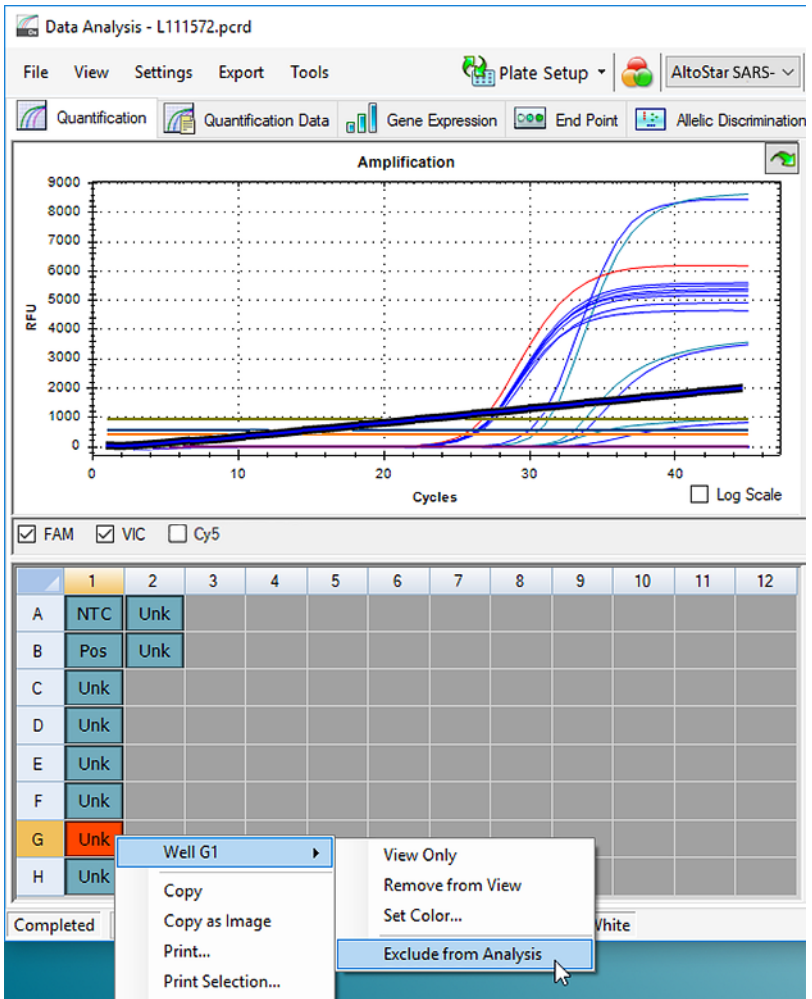

Figure 37: Data Analysis window: Exclude Well from Analysis

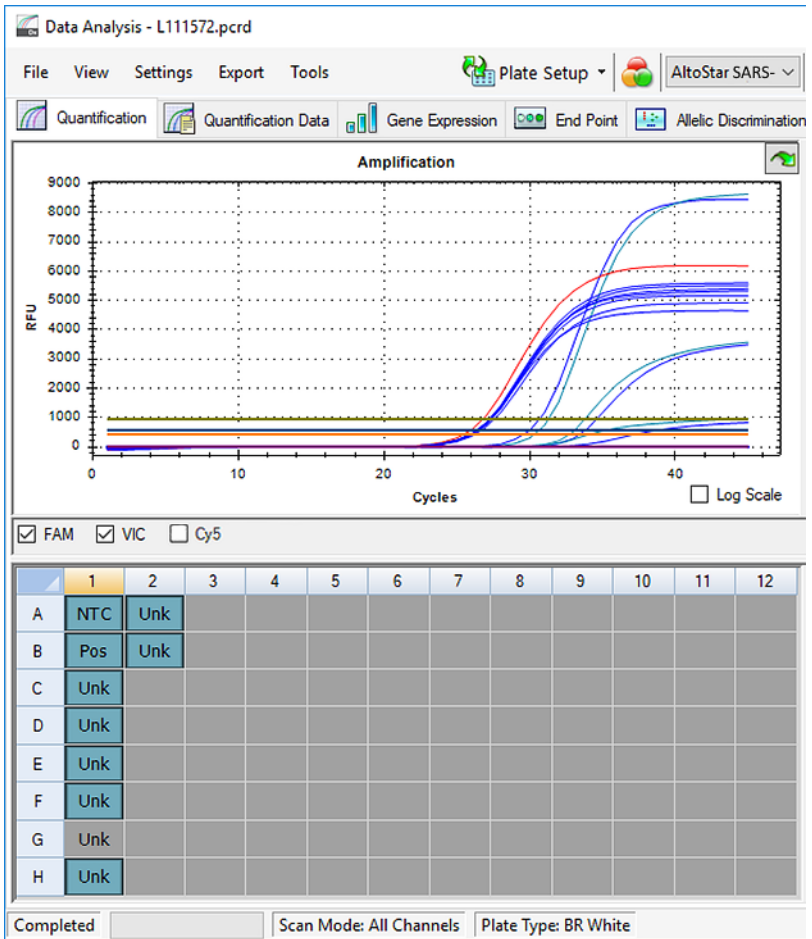**4.** The selected well is excluded from the analysis. No results will be generated for this well (see Figure 38).

Figure 38: Data Analysis window: Excluded well

8.20.3 Setting of Thresholds

The thresholds for the FAM™ (E gene), the Cy5 (S gene) and the VIC™ (Internal Control) detection channels have to be set manually by the user according to the signals of the controls.

1. In the Data Analysis Window (see Figure 29) ensure to select the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see Figure 34) of the toolbar.

2. At the left side of the **Data Analysis** window tick only the **VIC** checkbox for the detection channel of the Internal Control (see Figure 39).
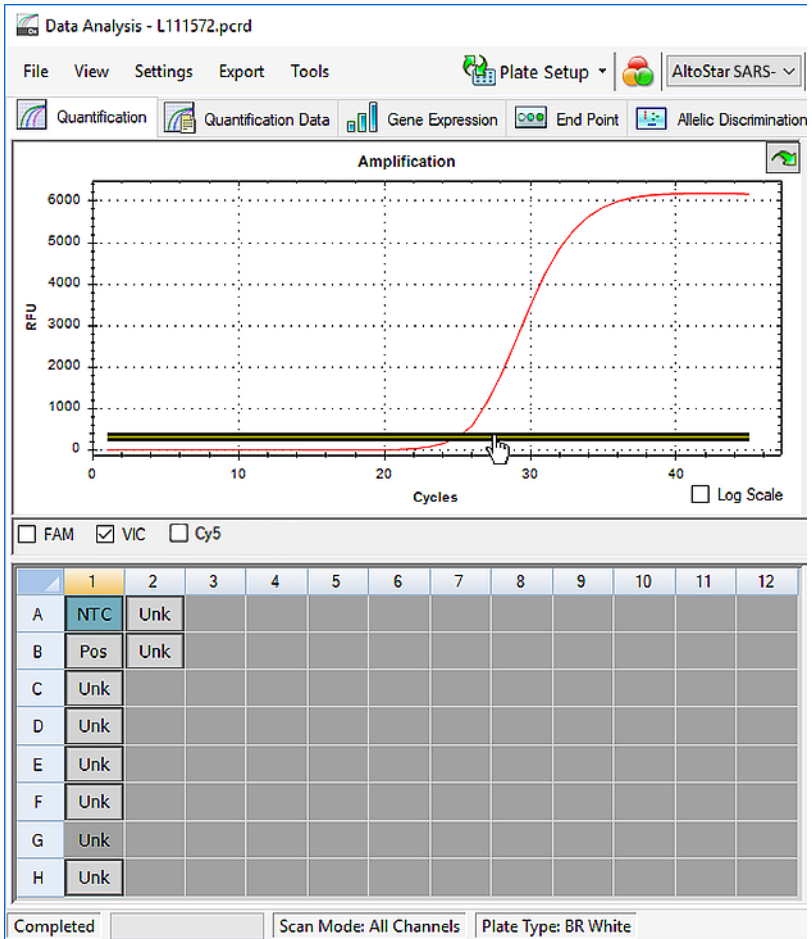
Figure 39: Data Analysis window: Setting the VIC™ threshold

3. Select only the NTC well in the plate view of the **Data Analysis** window (see Figure 39).

4. Drag the threshold into the exponential area of the NTC signal (see Figure 39).

NOTE

ℹ The NTC contains Internal Control Template, which leads to an Internal Control 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 E gene (see Figure 40).
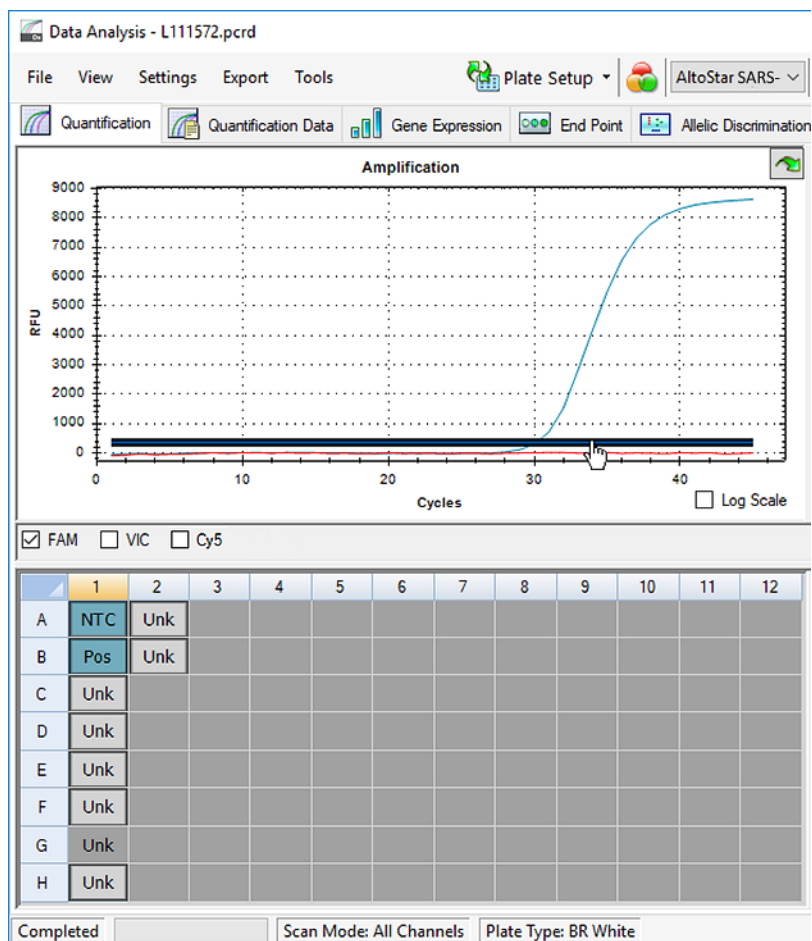

Figure 40: Data Analysis window: Setting the FAM™ threshold

6. Select only the wells containing the NTC and the Positive Control in the plate view of the **Data Analysis** window (see Figure 40).

7. Drag the threshold well above the signal of the NTC into the exponential area of the Positive Control signal.

8. For the detection channel of S gene untick the **FAM** checkbox at the left side of the Data Analysis window and tick the **Cy5** checkbox, respectively and repeat steps 6 and 7.

8.20.4 Exclusion of Wells

1. In the Data Analysis window (see Figure 29) ensure to select the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group button** (see Figure 34) of the toolbar.

2. Identify all wells to be exluded.

3. Click each well to be exluded with the right mouse button and select **Well... → Exclude from Analysis** (see Figure 41 and Figure 42).
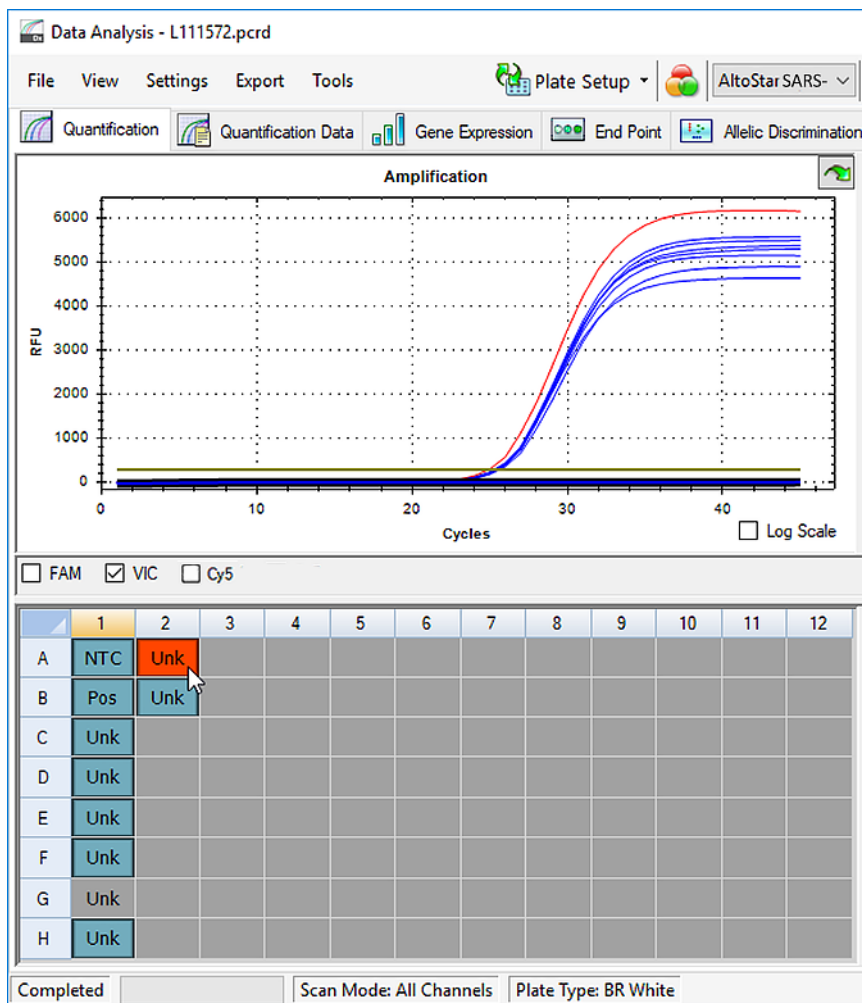
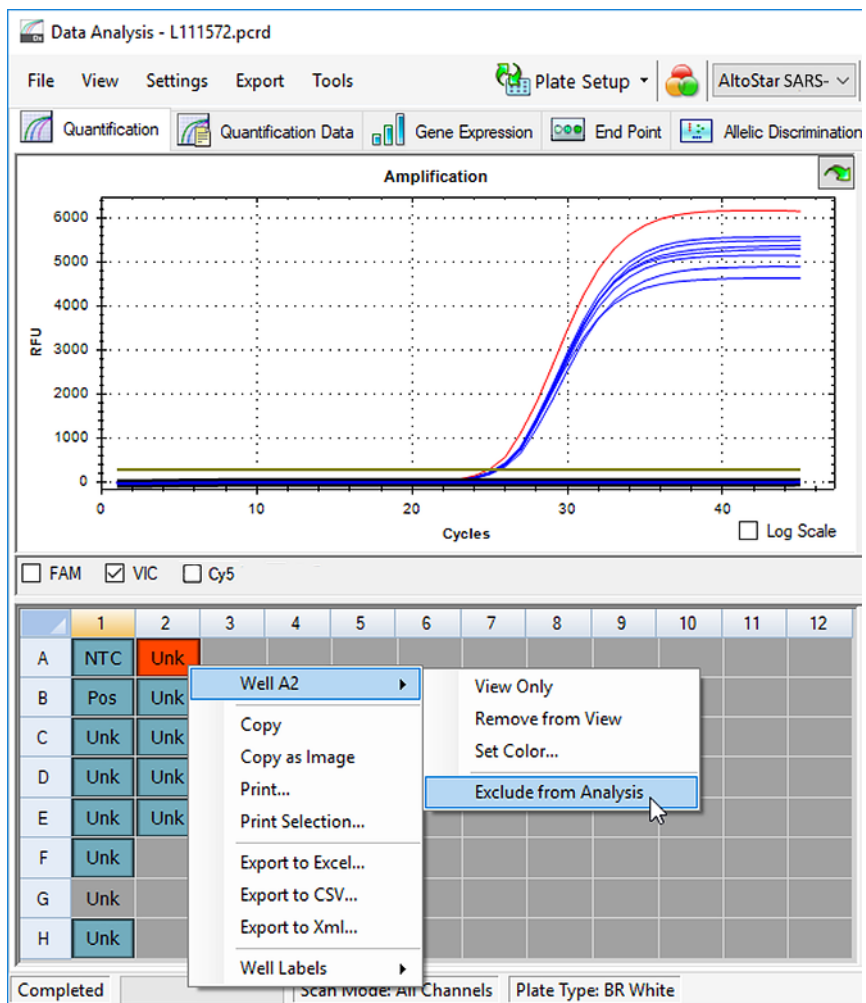**Figure   41**: Data Analysis window: Well to be excluded

Figure 42: Data Analysis window: Exclude Well from Analysis

The selected well is excluded from the analysis. No results will be generated for this well (see Figure 43).
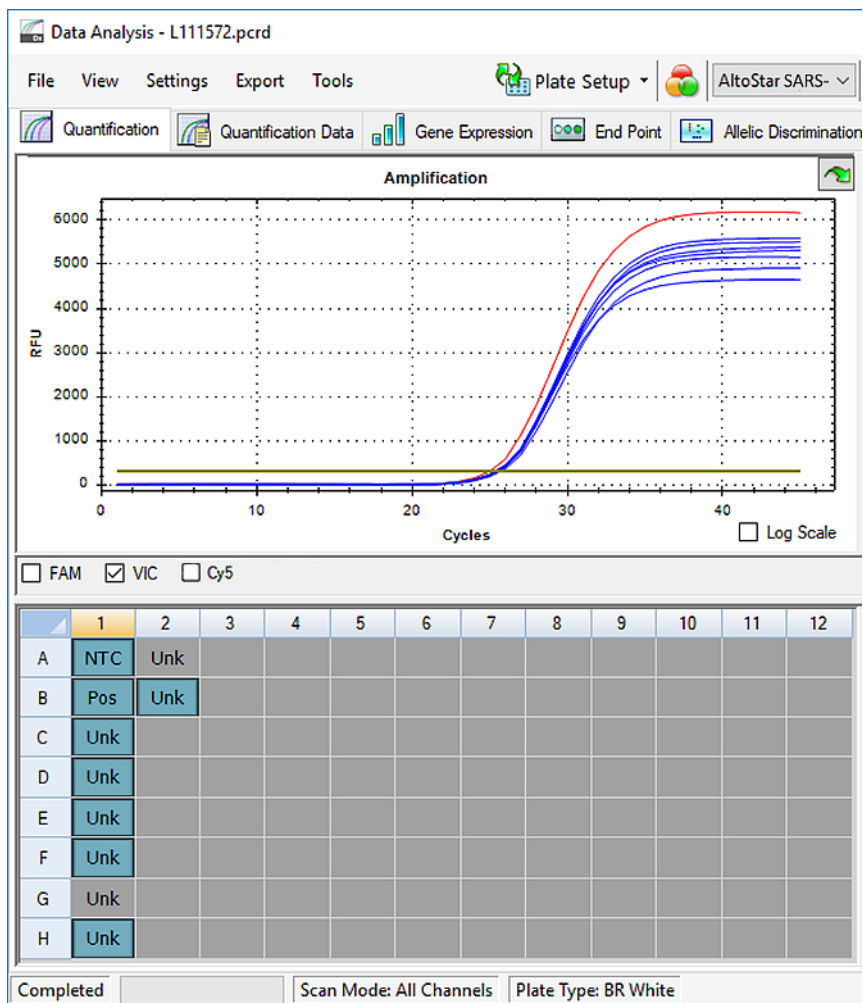

Figure 43: Data Analysis window: Excluded Well

8.20.5 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 (see Figure 29) ensure to select the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see Figure 34) of the toolbar.

2. Ensure that all steps of the analysis process (see chapters 8.20.1 Baseline Correction to 8.20.3 Setting of Thresholds) have been completed for the **Well Group** of the AltoStar® SARS-CoV-2 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 (see Figure 44).
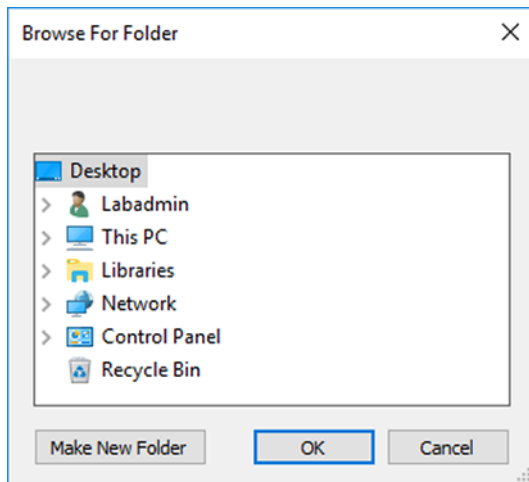

Figure 44: Browse For Folder dialog

4. In the **Browse For Folder** dialog specify the location for the LIMS Result Files to be generated and click **OK**.

> **NOTE**
>
> **i** The LIMS Integration has to be implemented according to the specifications of altona Diagnostics. For information regarding LIMS integration, contact altona Diagnostics Technical Support (see chapter 15 Technical Assistance).

> **NOTE**
>
> **i** 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.

8.20.6 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 (see Figure 29) ensure to select the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see Figure 34) of the toolbar.

2. At the left side of the **Data Analysis** window tick the **VIC** checkbox as well as the **FAM** and **Cy5** checkbox.

3. Ensure that all steps of the analysis process (see chapters 8.20.1 Baseline Correction to 8.20.3 Setting of Thresholds) have been completed for the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5.

4. In the menu bar of the **Data Analysis** window click **Tools → Reports...** to open the **Report** dialog (see Figure 45).
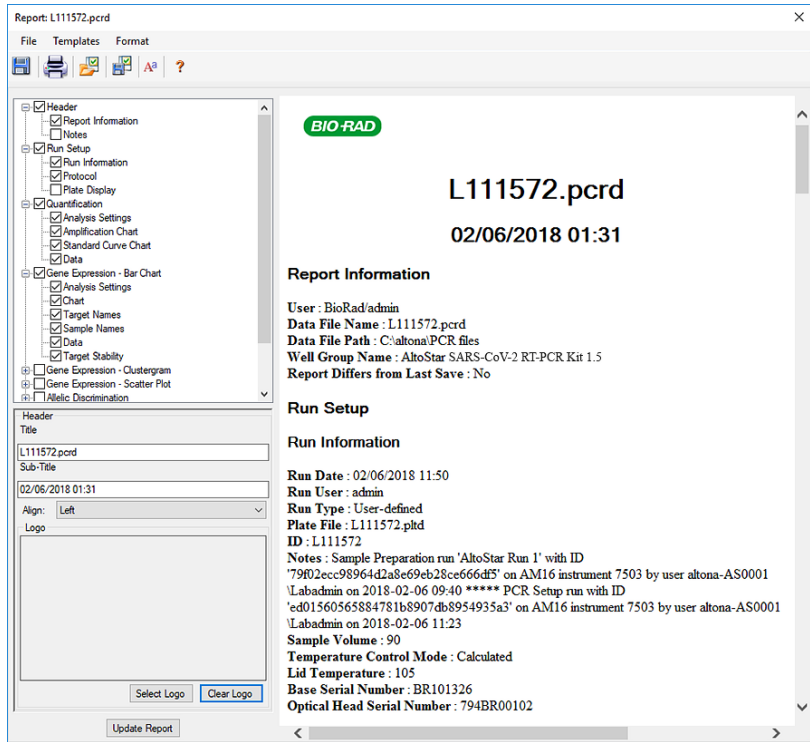
Figure   45: Report dialog: Export of the results as Report

5. Ensure that at least the following content is selected for report generation in the upper left part of the **Report** dialog (see Figure 46):

☑ Header
　　☑Report Information
☑ Experiment Setup
　　☑Run Information
　　☑Protocol
☑ Quantification
　　☑Analysis Settings
　　☑Amplification Chart
　　☑Standard Curve Chart
　　☑Data

Figure 46: Report Dialog

Select or deselect additional content of the **Report** by ticking the respective check boxes as required.

6. In the menu bar of the **Report** dialog click **File → Save As...** to open the **Save Report** dialog.

7. In the **Save Report** dialog specify the name and location for the report file to be generated and click **Save**.

8.20.6.1 Manual Interpretation of Results

1. Open the **Report** file generated for the **Well Group** of the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5 (see chapter 8.20.6 Export of PCR Results for Manual Result Interpretation).

2. Refer to the **Quantification Data** table in the **Report** (see Figure 47). The table comprises four rows for each **Sample** - one each for the **Target** *E gene, S gene* and one for the **Target** *Internal Control.*

Quantification Data

Well	Fluor	Target	Content	Sample	Biological Set Name	Cq	Cq Mean	Cq Std. Dev	Well Note
A01	Cy5	S gene	NTC	NTC | 131119012004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	
B01	Cy5	S gene	Pos Ctrl	SARS-CoV-2 PC | 131119012004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	29.25	29.25	0.000	
B02	Cy5	S gene	Unkn	Sample 8 | 00000008	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
C01	Cy5	S gene	Unkn	Sample 1 | 00000001	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
D01	Cy5	S gene	Unkn	Sample 2 | 00000002	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
E01	Cy5	S gene	Unkn	Sample 3 | 00000003	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
F01	Cy5	S gene	Unkn	Sample 4 | 00000004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
H01	Cy5	S gene	Unkn	Sample 6 | 00000006	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
A01	FAM	E gene	NTC	NTC | 131119012004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	
B01	FAM	E gene	Pos Ctrl	B-ßCoV PC | 131119012004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	30.07	30.07	0.000	
B02	FAM	E gene	Unkn	Sample 8 | 00000008	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
C01	FAM	E gene	Unkn	Sample 1 | 00000001	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	29.25	29.25	0.000	qualitative
D01	FAM	E gene	Unkn	Sample 2 | 00000002	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
E01	FAM	E gene	Unkn	Sample 3 | 00000003	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
F01	FAM	E gene	Unkn	Sample 4 | 00000004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
H01	FAM	E gene	Unkn	Sample 6 | 00000006	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	N/A	0.00	0.000	qualitative
A03	VIC	Internal Control	NTC	NTC | 131119012004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	24.91	24.91	0.000	
B02	VIC	Internal Control	Unkn	SARS-CoV-2 PC | 131119012004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	25.30	25.30	0.000	qualitative
C01	VIC	Internal Control	Unkn	Sample 1 | 00000001	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	25.34	25.34	0.000	qualitative
D01	VIC	Internal Control	Unkn	Sample 2 | 00000002	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	25.45	25.45	0.000	qualitative
E01	VIC	Internal Control	Unkn	Sample 3 | 00000003	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	25.27	25.27	0.000	qualitative
F01	VIC	Internal Control	Unkn	Sample 4 | 00000004	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	25.38	25.38	0.000	qualitative
H01	VIC	Internal Control	Unkn	Sample 6 | 00000006	AltoStar SARS-CoV-2 RT-PCR Kit 1.5	25.40	25.40	0.000	qualitative

Figure 47: Report: Quantification Data

Qualitative results would be marked by the term *qualitative* in the **Well Note** column of the **Quantification Data** table (see Figure 47).

3. In that case, identify each row with the **Target** *E gene* or *S gene* and the term *qualitative* in the **Well Note** column.
4. In these rows, refer to the C_q column for the result of the respective **Sample**.
5. Refer to Table 9 for interpretation of results.

Table 9: Result Interpretation

Detection Channel			Result Interpretation
FAM™ (E gene)	**Cy5 (S gene)**	**VIC™ (Internal Control)**	
+	+	+*	Lineage B-βCoV and SARS-CoV-2 specific RNA detected.
+	-	+*	Only lineage B-βCoV specific RNA detected.[1,2]
-	+	+*	Only SARS-CoV-2 specific RNA detected.[1]
-	-	+	Neither lineage B-βCoV nor SARS-CoV-2 specific RNA detected. The sample does not contain detectable amounts of lineage B-βCoV or SARS-CoV-2 specific RNA.
-	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the VIC™ detection channel is not required for positive results either in the FAM™ detection channel or in the Cy5 detection channel. A high lineage B-βCoV (target E gene) and/or SARS-CoV-2 (target S gene) RNA load in the sample can lead to reduced or absent Internal Control signals.

[1] Detection in only one of the two respective detection channels for E gene and S gene might be due to low viral RNA concentration or due to mutation of one of the two target sequences.

[2] Sample may be re-tested by repeating the extraction and RT-PCR. If the repeated result remains discrepant then additional confirmatory testing may be conducted.

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.

NOTE

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

NOTE

Dispose of hazardous and biological waste only in compliance with local and national regulations to avoid environmental contamination.

NOTE

Liquid waste and any liquids containing Lysis Buffer or Wash Buffer 1 contain guanidine thiocyanate, which can form toxic, highly reactive and volatile compounds when combined with bleach or strong acids.

NOTE

The PCR Plate must be disposed of in sealed state, since the AltoStar® PCR Plate Sealing Foil cannot be removed.

10. Technical Assistance

For customer support, please contact the altona Diagnostics Technical Support:

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

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

11. Trademarks and Disclaimers

AltoStar® (altona Diagnostics); CFX96™, CFX Manager™, Hard-Shell® (Bio-Rad); 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.

For research use only (RUO)! Not for use in diagnostic procedures.

12. Explanation of Symbols

Symbol	Explanation
RUO	Research Use Only
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
MAT	Material number
	Version
i	Note

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