

Instructions for Use

AltoStar[®] Purification Kit 1.5

11/2018 EN

AltoStar[®]

Purification Kit 1.5

For research use only!

(RUO)



PK15-06



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Content

1.	About these Instructions for Use	6
2.	Kit Content.....	6
3.	Storage and Handling	8
3.1	Storage.....	8
3.2	Handling	8
4.	Product Description.....	9
4.1	Principle of Method.....	9
5.	Samples	12
5.1	Sample Types.....	12
5.2	Sample Volume	12
5.3	Sample Tubes.....	12
5.4	Sample Barcodes	14
6.	Consumables and Additional Material Needed	15
7.	Warnings and Precautions	17
8.	Procedure	19
8.1	Overview.....	19
8.1.1	Sample Preparation.....	21
8.1.1.1	Plasma, Serum, CSF, Urine and Swabs in Transport Medium...20	
8.1.1.2	Whole Blood.....	21
8.1.1.3	Stool	22
8.1.2	Preparing Reagents for a Purification Run.....	22
8.2	During the Purification Run.....	23
8.3	Purification Run Results	23
8.4	Sealing of the Eluate Plate	24

8.5	Unsealing of the Eluate Plate	24
8.6	Storage Reagents after Use	24
9.	Disposal	24
10.	Troubleshooting Guide	25
11.	Trademarks and Disclaimers	27
12.	Explanation of Symbols	28

1. About these Instructions for Use

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.

NOTE



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

Application: The AltoStar® Purification Kit 1.5 uses magnetic particle technology and allows the isolation and purification of nucleic acids from biological specimens in conjunction with the AltoStar® Automation System AM16 (Hamilton; in the following summarized as AltoStar® AM16) and the AltoStar® Internal Control 1.5.

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

2. Kit Content

The AltoStar® Purification Kit 1.5 is shipped in two separate boxes **Box 1** and **Box 2** (see Table 1 and Table 2).

Table 1: Kit content **Box 1**

Component	Number per Box	Volume per Container [ml]
Lysis Buffer	6	190
Wash Buffer 1	6	175
Wash Buffer 2	6	175
Wash Buffer 3	6	175
Whole Blood Pretreatment Buffer	8	58
Container Re-Sealing Foil	120	n.a.

Table 2: Kit content **Box 2**

Component	Number per Box	Volume per Tube [ml]
Enhancer	24	1.2
Magnetic Beads	24	1.5
Elution Buffer	12	8.2

The AltoStar® Purification Kit 1.5 contains reagents sufficient for 1152 sample purifications when using 500 µl sample volume only or for 576 sample purifications when using 1000 µl sample volume only.

Upon receipt please check the product and its components for:

- Integrity
- Completeness with respect to number, type and filling
- Correct labelling

If any kit component has been compromised during shipment or is missing, contact altona Diagnostics Technical Support for assistance.

3. Storage and Handling

3.1 Storage

The AltoStar® Purification Kit 1.5 is shipped at room temperature. **Box 1** has to be stored at +15 °C to +30 °C and **Box 2** has to be stored at +2 °C to +8 °C upon receipt (see Table 3).

CAUTION



Store the reagent containers and tubes in an upright position.

Table 3: Storage conditions for **Box 1** and **Box 2**

Storage Conditions	
Box 1	Box 2
+15 °C to +30 °C	+2 °C to +8 °C

3.2 Handling

All reagents included in the AltoStar® Purification Kit 1.5 are ready-to-use solutions. They are stable after initial opening for 14 days, when closed after each use and stored as follows: Magnetic Beads, Enhancer and Elution Buffer should be closed with the original cap after use and stored at +2 °C to +8 °C. The Lysis Buffer and Wash Buffer 1, 2 and 3 should be resealed after use with unused Container Re-Sealing Foil and stored at +15 °C to +30 °C. The Whole Blood Pretreatment Buffer should be closed with the original cap after use and stored at +15 °C to +30 °C.

NOTE



The Lysis Buffer can form precipitates at low temperature or after prolonged storage. If precipitates are visible in the liquid, heat the Lysis Buffer (≤ 50 °C, e.g. in a water bath) before use with careful intermittent pivoting until the precipitates are completely dissolved.

4. Product Description

Table 4: Kit component description

Kit Component	Description
Lysis Buffer	The Lysis Buffer contains chaotropic salts and surfactants (guanidine thiocyanate, Octoxynol 9) to disrupt cells or virions chemically. It stabilizes nucleic acids and protects them against nucleases in solution.
Wash Buffer 1	The Wash Buffer 1 contains different salts and organic solvents (guanidine thiocyanate and ethanol) to remove proteins and other impurities.
Wash Buffer 2	The Wash Buffer 2 contains organic solvents (ethanol) to remove proteins and other impurities.
Wash Buffer 3	The Wash Buffer 3 contains different salts in order to purify the nucleic acids.
Whole Blood Pretreatment Buffer	The Whole Blood Pretreatment Buffer contains surfactants and salts to stabilize and liquefy whole blood samples for automated processing on the AltoStar® AM16.
Enhancer	The Enhancer stabilizes and protects nucleic acids against nucleases in solution.
Magnetic Beads	The Magnetic Beads are coated with a thin layer of silica to bind free nucleic acids in solution. The magnetic characteristic allows the separation of beads from liquids in a magnetic field.
Elution Buffer	The Elution Buffer is a low salt buffer to release the nucleic acids from the Magnetic Beads for subsequent analysis.
Container Re-Sealing Foil	The Container Re-Sealing Foil is an adhesive tape seal to be used for resealing the containers of the AltoStar® Purification Kit 1.5 (Lysis Buffer and Wash Buffer 1, 2, and 3) after use.

4.1 Principle of Method

The AltoStar® Purification Kit 1.5 allows the isolation and purification of RNA and DNA from biological specimens in conjunction with the AltoStar® AM16 and the AltoStar® Internal Control 1.5.

The purification procedure comprises 3 automated steps on the AltoStar® AM16 (see Figure 1).

1. In the first step nucleic acids are released by chemical and mechanical lysis under chaotropic high salt conditions. The conditions stabilize the nucleic acids in solution and enable their binding to the magnetic silica beads. The AltoStar® Internal Control 1.5 is automatically added by the AltoStar® AM16.
2. In the following washing steps different wash buffers are used to remove proteins and other impurities.
3. Finally the nucleic acids are released from the magnetic beads with an elution buffer and transferred to the eluate plate.

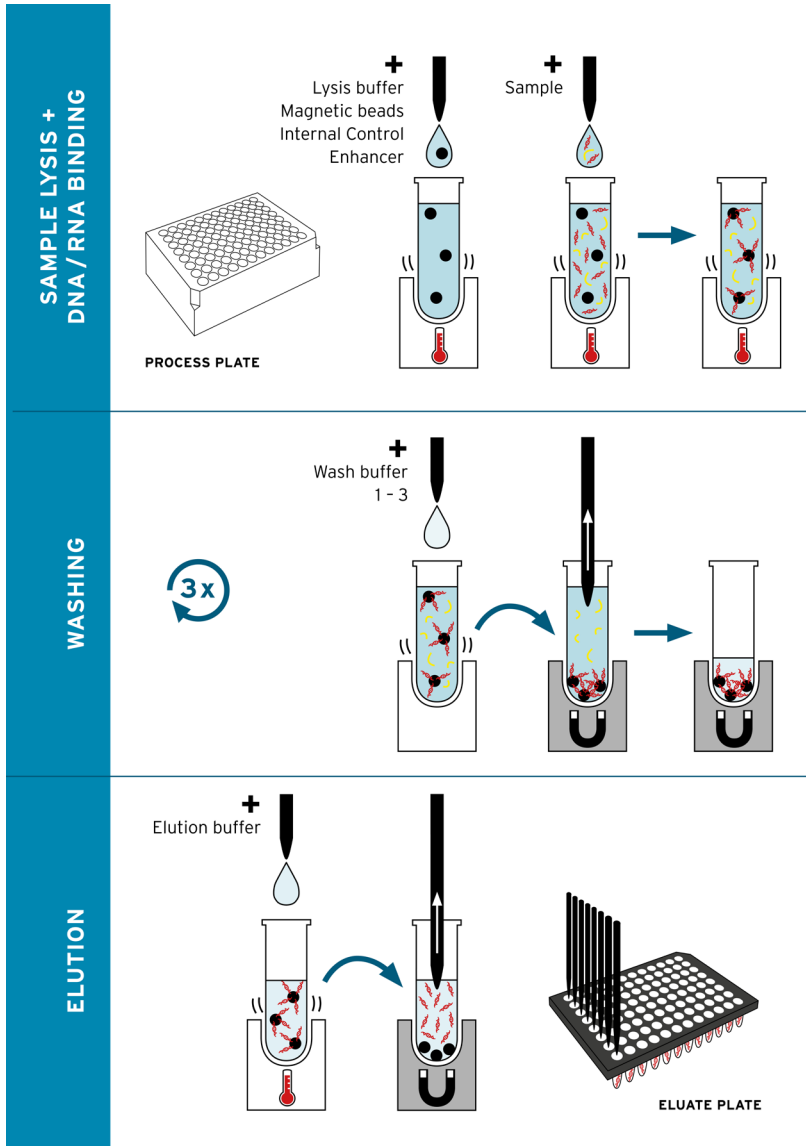


Figure 1: Illustration of the AltoStar® purification procedure

5. Samples

5.1 Sample Types

The following sample types can be used with the AltoStar® Purification Kit 1.5:

- Whole blood
- Plasma
- Serum
- Urine
- Stool
- Cerebrospinal fluid (CSF)
- Swabs in transport medium
 - Calcium alginate swabs, swabs with wooden shafts and/or cotton tips as well as bacteriological swabs containing jelly agar should not be used.

NOTE



When working with frozen samples, samples should be completely thawed and properly mixed before use.



Different sample types can be processed simultaneously in one Purification Run with the AltoStar® Purification Kit 1.5.

5.2 Sample Volume

The AltoStar® Purification Kit 1.5 allows purification of either 500 µl or 1000 µl of a sample. Additional sample volume has to be provided to account for the dead volume of the sample tube used (see chapter 6.3: Sample Tubes).

5.3 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, EA05088).

Sample tubes that fulfill the following requirements can be tested for applicability:

- Height below 100 mm
- Inner diameter greater than 9 mm
- Outer diameter within 11 – 14 mm when using the Tube Carrier 32
- Outer diameter within 14.5 – 18 mm when using the Tube Carrier 24

Depending on the chosen sample volume, 500 µl or 1000 µl of sample are automatically transferred from the sample tube to the purification process. To account for the dead volume of the sample tube additional sample volume has to be provided. The necessary excess volume depends on the tube geometry.

The volumes specified in Table 5 can be used as a starting point for testing sample tube and dead volume suitability.

Table 5: Suggested total sample volumes for different tube types

Outer tube diameter [mm]	Total volume [µl] needed for 500 µl / 1000 µl processing volume		
	Round Bottom	Flat Bottom	Conical Bottom
11	Not suitable	900 / 1400	Not suitable
11.5	700 / 1300	900 / 1400	700 / 1300
12	700 / 1300	900 / 1400	900 / 1400
13	700 / 1300	900 / 1400	1000 / 1500
14	800 / 1300	900 / 1400	1000 / 1500
15	1300 / 1900	900 / 1400	1000 / 1500
15.3	1300 / 1900	1600 / 2200	1000 / 1500
16	1300 / 1900	1600 / 2200	1000 / 1500
16.5	1400 / 1900	1700 / 2200	1000 / 1500
16.8	1500 / 1900	Not tested	1000 / 1500
17	1500 / 1900	Not tested	1000 / 1500
18	1500 / 1900	Not tested	Not tested

5.4 Sample Barcodes

For automated sample identification by the AltoStar® AM16 sample tubes must be labelled with a suitable barcode (see Figure 2).

For a given Purification Run, ensure that each sample barcode is unique. The sample barcode must contain between one and twenty characters. It is possible to use numbers (0 - 9) and letters (A - Z, a - z).

The barcode label must be fixed to the tube within a range of 20 mm to 100 mm from the bottom of the tube.

The label must fit tightly at an angle of approximately 90° to the tube. The label must fit tightly over its whole length.

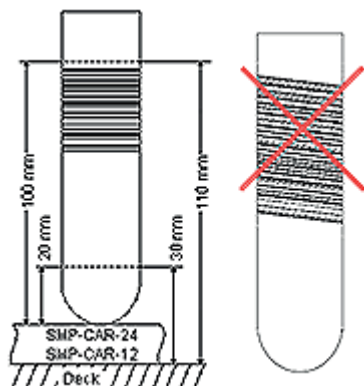


Figure 2: Placement of barcode on the sample tube

6. Consumables and Additional Material Needed

Table 6: Consumables

Material Name	Description	Order No.	Shipping Unit
AltoStar® Processing Plate	AltoStar® Processing Plate	EA05079	50
FrameStar® Eluate Plate*	FrameStar® Eluate Plate*	EA05081	50
AltoStar® Eluate Plate Sealing Foil	AltoStar® Eluate Plate Sealing Foil with cut corner A12 and 10 mm sides	EA05082	100
1000 µl CO-RE Tips	CO-RE Tips, 8 x 480 High Volume Tips (1000 µl) with Filters	EA05085	3840
300 µl CO-RE Tips EA05086	CO-RE Tips, 12 x 480 Standard Volume Tips (300 µl) with Filters	EA05086	5760
Waste Bag	Sterilbag, Bag Type 60 - Autoclave 134 °C	EA05087	500

* FrameStar® is covered by one or more of the following US patents or their foreign counterparts, owned by Eppendorf AG: US Patent Nos. 7,347,977 and 6,340,589. FrameStar® is a registered trademark owned by 4titude® Ltd.

CAUTION



Do not substitute the material that is specified in Table 6. Use of other material can lead to damage to the AltoStar® AM16.

Table 7: Additional laboratory material and devices

Material	Description	Order No.	Shipping Unit
Plate Sealer	e.g. AltoStar® Plate Sealer	EA05149	1
Suitable sample tubes	e.g. 7 ml tube with cap, 82 x 13 mm	EA05088	1000
Sample tube caps	e.g. ribbed plug (<i>Lamellenstopfen</i>) PE, Ø 13 mm with high grip, for sample tubes	EA05089	2500

General material and devices

- Vortex mixer
- Powder-free gloves (disposable)
- Centrifuge for pretreatment of samples
- Centrifuge for centrifugation of plates
- Pipettes (adjustable, for sample preparation)
- Pipette tips with filters (disposable, for sample preparation)
- Sodium Chloride Solution (0.9 %)

7. Warnings and Precautions

Whole Blood Pretreatment Buffer



GHS05

H314 Causes severe skin burns and eye damage

H318 Causes serious eye damage

Contains:

Sodium hydroxide (CAS 50-01-1) 1 - 2 %

4-Nonylphenol (CAS 127087-97-0) 0.1 - 1 %

Lysis Buffer



GHS05

H302+H312+H332 Harmful in contact with skin or if inhaled or swallowed

H314 Causes severe skin burns and eye damage

H318 Causes serious eye damage

H412 Harmful to aquatic life with long lasting effect

EUH032 Contact with acids liberates very toxic gas

EUH071 Corrosive to the respiratory tract

Contains:

Guanidine thiocyanate (CAS 593-84-0) 50 - 70 %

Octoxinol (CAS 9036-19-5) 2.5 - 5 %

4-Morpholineethanesulfonic (CAS 4432-31-9) 1 - 2.5 %

4-Nonylphenol (CAS 127087-87-0) 0.1 - 1 %



GHS07

Wash Buffer 1



GHS05

H226 Flammable liquid and vapour

H314 Causes severe skin burns and eye damage

H318 Causes serious eye damage

H412 Harmful to aquatic life with long lasting effect

EUH032 Contact with acids liberates very toxic gas

EUH071 Corrosive to the respiratory tract

Contains:

Guanidine thiocyanate (CAS 593-84-0) 25 - 50 %

Ethanol (CAS 64-17-5) 25 - 50 %



GHS02

Wash Buffer 2



GHS07

H225 Highly flammable liquid and vapour

H319 Causes serious eye irritation

Contains:

Ethanol (CAS 64-17-5) 50 - 70 %



GHS02

Enhancer

GHS05

H314 Causes severe skin burns and eye damage

H318 Causes serious eye damage

Contains:

Tris(2-carboxyethyl)phosphine (CAS 51805-45-9) 10 - 20 %

NOTE

For more information, please consult the respective safety data sheets (SDSs).

- Observe the following measures when handling components of the AltoStar® Purification Kit 1.5:
 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. Do not smoke.
 - Wear protective gloves, protective clothing and eye protection.
 - IF SWALLOWED: Call a POISON CENTER or doctor/physician.
 - IF ON SKIN: Wash with plenty of water.
 - IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or doctor/physician if you feel unwell.
 - IF IN EYES: Rinse cautiously with water for several minutes and remove contact lenses if present and easy to do. Continue rinsing.
 - If skin irritation occurs or eye irritation persists: Get medical advice/attention.
 - Do not add bleach or acidic solutions directly to the sample preparation waste and any liquids containing Lysis Buffer or Wash Buffer 1. These buffers contain guanidine thiocyanate, which can form toxic, highly reactive and volatile compounds when combined with bleach or strong acids. If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water.
- Always wear a suitable lab coat, disposable gloves, and eye protection, when working with product components.

8. Procedure

8.1 Overview

The steps of the AltoStar® purification procedure are summarized in the following. For details refer to the user manual of the AltoStar® AM16 and of the AltoStar® Connect software (Hamilton).

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 sample data (sample type, sample volume,...)
- » Click the **Create Run** button in the tool bar to create the AltoStar® Run.

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 reagents to be loaded have the same *Loading Number* (not necessary for the AltoStar® Internal Control 1.5) and are not expired.
 - If precipitates are visible in the Lysis Buffer, heat it (≤ 50 °C) until it is 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 8.1.1 Sample Preparation.
- » 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 **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.
- » View the Purification Run results to confirm successful processing of each sample.

8.1.1 Sample Preparation

For successful nucleic acid isolation and purification the specifications regarding sample volume, sample type, sample tube and sample barcode as well as sample preparation should be followed carefully.

NOTE



At least 500 µl or 1000 µl sample volume should be provided, respectively, plus the required dead volume in a suitable sample tube. The sample volume is not checked by the system prior to processing. Short samples will not be processed and error flagged during the sample transfer step.



The samples should 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.

8.1.1.1 Plasma, Serum, CSF, Urine and Swabs in Transport Medium

Plasma, serum, cerebrospinal fluid (CSF), urine samples and swabs in transport medium can be processed without pretreatment.

CAUTION



Remove the swab before loading the sample tube on the AltoStar® AM16.

8.1.1.2 Whole Blood

1. Transfer the required volume of Whole Blood Pretreatment Buffer to a suitable sample tube and add the same volume of whole blood (free of solids and high-viscosity constituents) to achieve a volumetric ratio of 1:1.
2. Immediately and thoroughly mix by vortexing for 10 seconds. Insufficient mixing may render the sample unsuitable for processing due to increased viscosity or clotting.
3. Take care to avoid formation of bubbles. If bubbles have formed during mixing they can be removed after 2 - 3 minutes by carefully tapping the sample tube. Do not centrifuge the sample.

NOTE

The Purification Run on the AltoStar® AM16 for the pretreated Whole Blood samples should be started within 60 minutes from the beginning of the pretreatment. Prolonged incubation or insufficient mixing during preparation may render the sample unsuitable for processing due to increased viscosity or clotting.

8.1.1.3 Stool

1. Add 1 volumetric part stool to 25 volumetric parts 0.9 % sodium chloride solution (not provided).
2. Thoroughly mix by vortexing to achieve a homogenous suspension.
3. Centrifuge at 500 x g for 1 minute. Transfer the solids-free supernatant to a suitable sample tube.

8.1.2 Preparing Reagents for a Purification Run

1. Prepare sufficient amounts of AltoStar® Purification Kit 1.5 reagents with 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.

NOTE

For your convenience, the four digit Loading Number is displayed on the outside of each component box.

NOTE

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 colour changes may occur to the Lysis Buffer. These slight changes in colour do not indicate a change in the quality of the buffer.

3. Vortex the Magnetic Beads 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.

8.2 During the Purification Run

No further user interaction is required until the Purification Run has finished.

CAUTION



Do not push or pull carriers or the door of the AltoStar® AM16 during a Purification Run as this may abort the run.



Aborting the Purification Run after the Loading Complete dialog is confirmed will void the AltoStar® Run, preventing a restart.

1. Close reagent tubes with the appropriate tube caps.
2. Close reagent containers with unused Container Re-Sealing Foils.
3. Store reagents for reuse as described in chapter 3 Storage and Handling.

8.3 Purification Run Results

Table 8: Purification Run Results

Status	Purification Run Result
Processed	The sample was successfully processed in the Purification Run.
Error	The sample was not processed successfully. No eluate of this sample is available.

Two Purification Run result files containing detailed information regarding the Purification Run results are automatically generated by the AltoStar® Connect software, a (.xml) file and a (.pdf) file.

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

8.4 Sealing of the Eluate Plate

In case the eluates in the Eluate Plate are to be stored, the plate should be sealed with AltoStar® Eluate Plate Sealing Foil.

CAUTION



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.

8.5 Unsealing of the Eluate Plate

1. Remove the AltoStar® Eluate Plate Sealing Foil from the Eluate Plate as follows:
2. Briefly centrifuge the Eluate Plate in a plate centrifuge to remove any liquid from the inside of the sealing foil.
3. Press the Eluate Plate onto a table to avoid sudden plate movements during the removal of the sealing foil.
4. Start peeling in one corner and slowly and steadily pull the sealing foil towards the diagonally opposite corner until it is removed.

8.6 Storage Reagents after Use

Magnetic Beads, Enhancer and Elution Buffer should be closed with the original cap after use and stored at +2 °C to +8 °C. The Lysis Buffer and Wash Buffer 1, 2 and 3 should be resealed after use with unused Container Re-Sealing Foil and stored at +15 °C to +30 °C. The Whole Blood Pretreatment Buffer should be closed with the original cap after use and stored at +15 °C to +30 °C.

9. Disposal

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

CAUTION

Do not add bleach or acidic solutions directly to the sample preparation waste and any liquids containing Lysis Buffer or Wash Buffer 1. These buffers contain guanidine thiocyanate, which can form toxic, highly reactive and volatile compounds when combined with bleach or strong acids.

10. Troubleshooting Guide

Problem: Precipitate in reagent

Possible Cause	Suggestions
Storage of the Lysis Buffer container at low temperature or prolonged storage	If the Lysis Buffer container is already opened, make sure to reseal it with Container Re-Sealing Foil. Heat the Lysis Buffer container (≤ 50 °C, e.g. in a water bath) with careful intermittent pivoting until the precipitates are completely dissolved.
Excessive evaporation due to improper use and/or sealing may lead to increased salt concentration in reagents.	Discard the reagent. Make sure to immediately close the reagent containers with Container Re-Sealing Foil and reagent tubes with lids after use.

Problem: Low yield or purity of nucleic acid

Possible Cause	Suggestions
Storage of reagents under wrong conditions	Discard reagents. Make sure to store the product components under defined storage conditions (see chapter 3 Storage and Handling).

Possible Cause	Suggestions
Reagents were not closed and/or stored properly in between use.	Discard reagents. Make sure to store the product components under defined storage conditions (see chapter 3 Storage and Handling). Make sure to immediately close the reagent containers with Container Re-Sealing Foil and reagent tubes with lids after use.
Improper pretreatment of samples	Make sure to prepare samples according to the instructions in chapter 8.1.1 Sample Preparation.
Frozen samples were not thawed or mixed properly.	Make sure samples are completely thawed and properly mixed before use.
Incomplete sample lysis	Before use, check that the Lysis Buffer does not contain precipitates. If the Lysis Buffer container is already opened, make sure to reseal it with Container Re-Sealing Foil. Heat the Lysis Buffer container (≤ 50 °C, e.g. in a water bath) with careful intermittent pivoting until the precipitates are completely dissolved.

Problem: Un-processed sample

Possible Cause	Suggestions
High sample viscosity or solids in the sample	Make sure to prepare samples according to chapter 8.1.1 Sample Preparation.
Insufficient sample volume	Short samples will not be processed and error flagged during the sample transfer step. Make sure to provide the processing volume plus the required dead volume suitable for the sample tube used (see chapter 5.3 Sample Tubes).

Problem: Un-processed whole blood sample

Possible Cause	Suggestions
High sample viscosity due to prolonged incubation with Whole Blood Pretreatment Buffer.	Make sure to comply with the mixing requirements and to start the Purification Run on the AltoStar® AM16 within 60 minutes from the beginning of the pretreatment (see chapter: 8.1.1 Sample Preparation).

11. Trademarks and Disclaimers

















AltoStar® (altona Diagnostics GmbH), FrameStar® (4titude)

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! Not for use in diagnostic procedures.

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12. Explanation of Symbols

Symbol	Explanation
	Research Use Only
	Batch code
	Content
	Catalogue number
	Number
	Component
	Consult instructions for use
	Contains sufficient for "n" tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Material number
	Version
	Note
	Global Trade Item Number

Notes:

Notes:

always a drop ahead.

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