

Automation of the MACHEREY-NAGEL NucleoSpin® 96 RNA Kit on the ASSIST PLUS pipetting robot

Introduction

MACHEREY-NAGEL NucleoSpin® 96 nucleic acid extraction and purification kits are known to yield high quality and abundant nucleic acids. The 96 well silica membrane plate format used in the NucleoSpin 96 RNA kit is convenient for extracting RNA from cell cultures or tissues in high throughput laboratories, but the many pipetting steps required for manual processing of samples are tiring for

users, and introduce the possibility of pipetting errors at each step. Performing the assay on the ASSIST PLUS pipetting robot eliminates this possibility, and reduces the strain of repetitive pipetting by providing semi-automated sample extraction. The processing of 96 samples with the ASSIST PLUS pipetting robot is possible in less than 1 hour.

Key benefits:

- 96 samples can be extracted per run. If extraction of fewer samples is desired, programs may be modified. The ASSIST PLUS pipetting robot is equipped with a 12 channel VIAFLO electronic pipette, making the process even faster.
- An external plate shaker and MACHEREY-NAGEL Vacuum Manifold are placed directly on the deck of the ASSIST PLUS. This eliminates the need to transport plates from the liquid handler to these instruments and back.
- Manual interventions are limited to operating the on and off button of the shaker and vacuum, changing the reagent reservoir, and placing the elution plate in the vacuum manifold. Instructions for these interventions are provided on the pipette's bright screen, ensuring that the user does not lose track of the process.
- Benefits of the MACHEREY-NAGEL NucleoSpin 96 RNA kit over other kits include the addition of a DNA digestion step to ensure that the eluted RNA is free from DNA contamination, and the inclusion of a specially designed wash plate inserted into the manifold beneath the binding plate. This wash plate prevents cross contamination of samples during the vacuuming steps.

Overview: How to automate the MACHEREY-NAGEL NucleoSpin 96 RNA Kit

In this protocol, the pipetting steps of the MACHEREY-NAGEL NucleoSpin 96 RNA kit are automated on the ASSIST PLUS pipetting robot. With the NucleoSpin 96 RNA kit RNA can be isolated from cells or tissues (up to 2×10^6 cells or 30 mg tissue). All liquids are transferred automatically, with manual interventions only needed for turning the shaker and vacuum on and off, loading reagent reservoir 2, and preparing the elution plate. The ASSIST PLUS operates a 12 channel VIAFLO 1250 μ l electronic pipette with 1250 μ l Sterile, Filter GripTips.

Before beginning, RNase-free rDNase is reconstituted (see MACHEREY-NAGEL protocol), rDNase reaction mixture is prepared, and ethanol (96-100 %) is added to the wash buffers RA3 and RA4.

ASSIST PLUS



Experimental set-up

Deck Position A: 8 row reagent reservoir holding kit buffers.

Deck Position B: 96 well U-bottom, square, deep well plate placed on the INHECO Teleshake.

Deck Position C: NucleoVac 96 Vacuum Manifold with RNA Binding Plate and waste capture plate.

Important: Align the vacuum manifold before each run (see Appendix).



Figure 1: General set-up of the ASSIST PLUS pipetting robot for the RNA isolation program.

For the initial set-up, assemble the manifold as described in **Figure 2**, with the NucleoSpin 96 RNA Binding Filter Plate on top of the manifold and the Wash Plate in the manifold.

Overview of steps:

- Step 1:** Reagent transfer
- Step 2:** Lysis of cells
- Step 3:** Binding
- Step 4:** Desalting the silica membrane
- Step 5:** Digestion of DNA
- Step 6:** Washing
- Step 7:** Drying
- Step 8:** Elution

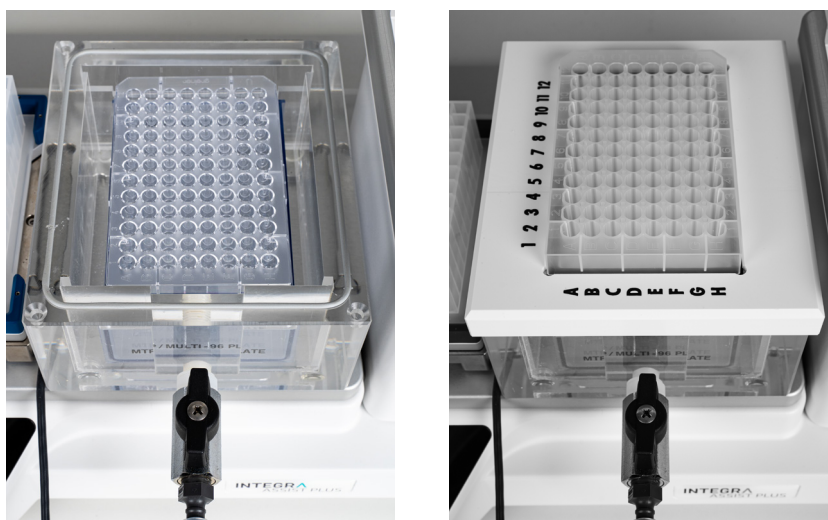
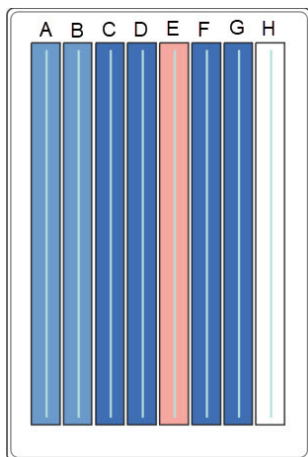


Figure 2: Vacuum manifold set-up for steps 3-7. Insert the 'MTP/MULTI-96 PLATE' spacers, waste container and the MN Wash Plate in the manifold. Place the manifold lid on top of the manifold base, and then place the NucleoSpin 96 RNA Binding Plate on top of the manifold.

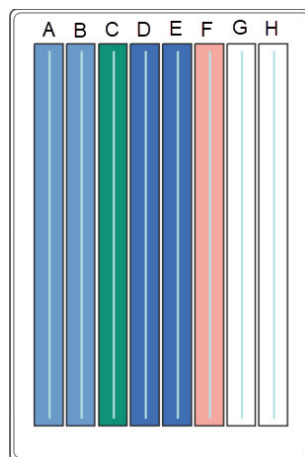
1. Reagent transfer

STEP: Transfer the previously prepared reagents.

HOW TO: Transfer the prepared reagents into two INTEGRA 8 row reagent reservoirs according to **Figure 3**.

**Reservoir 1**

Row A: 15 ml buffer RA1
 Row B: 15 ml buffer RA4
 Row C and D: 25 ml buffer RA3
 Row E: 10 ml rDNase
 Row F and G: 25 ml buffer RA2

**Reservoir 2**

Row A and B: 30 ml buffer RA3
 Row C: 20 ml buffer RA3
 Row D and E: 25 ml buffer RA4
 Row F: 10 ml of elution buffer

Figure 3: Preparation of two 8 row reagent reservoirs for MACHERY-NAGEL NucleoSpin 96 RNA extraction of cell culture on the ASSIST PLUS.

2. Lysis of cells

STEP: Lyse the cells by adding a solution containing chaotropic salt.

HOW TO: Place the 8 row reagent reservoir 1 with the buffers on **Position A**, the Teleshake with the samples on **Position B**, and the vacuum manifold on **Position C (Figure 4)**. Select and run the 'MN RNA CELL' VIALAB program. The ASSIST PLUS starts the program by transferring 130 µl of lysis buffer onto the plate holding samples. Turn on the shaker when indicated by the pipette and resuspend the cells by shaking at 1000 rpm. After a 4 minute delay, the pipette notifies the user to stop the shaker.

Tip:

- An air gap is introduced at the end of every dispense step to eliminate droplet build-up after the liquid dispense. Non-contact dispensing – avoiding the use of tip touch – prevents the risk of cross contamination. This post-dispense step ensures the accuracy of each dispense when using Repeat Dispense mode.

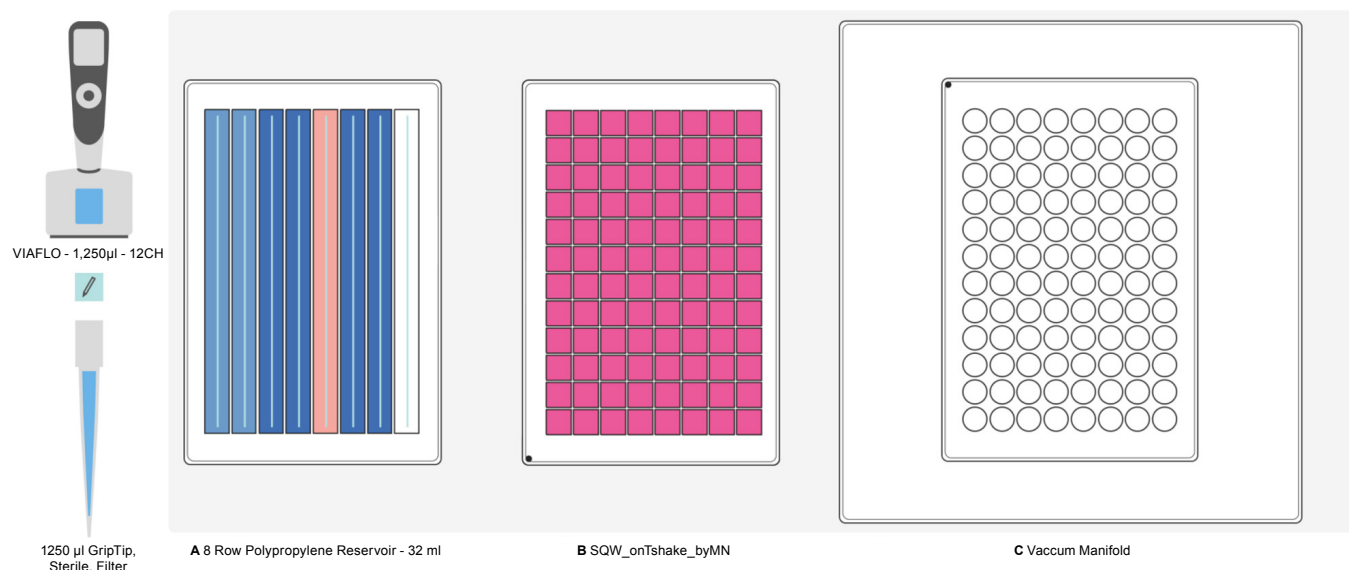


Figure 4: The initial deck set-up of the ASSIST PLUS for MACHEREY-NAGEL NucleoSpin 96 RNA isolation of cells.

Position A: Reservoir 1 containing the previously prepared buffers. **Position B:** INHECO Teleshake with 96 deep well plate holding samples. **Position C:** NucleoVac 96 Vacuum Manifold holding the binding plate.

3. Binding

STEP: Bind the RNA to the silica membrane.

HOW TO: After the cell lysis step, the ASSIST PLUS pipetting robot transfers 130 µl of buffer RA4 from row B of the reservoir to each well of the lysis plate.

IMPORTANT: Buffers RA1 and RA4 must be used in the same volume ratio.

The program automatically continues with the mixing step and the pipette transfers the crude lysate onto the NucleoSpin 96 RNA binding plate placed on the vacuum manifold. The pipette informs the user when to turn the vacuum pump on. Apply a vacuum of -0.2 bar and release it when all the lysate is bound to the silica membrane in the RNA binding plate, as indicated on the pipette.

Tip:

- The user is guided for each manual intervention by instructions appearing on the bright screen of INTEGRA's VIAFLO electronic pipette, ensuring that the operator does not lose track of which program step is in progress. The user should press RUN to continue the program after each intervention.

4. Desalting the silica membrane

STEP: Desalt the silica membrane by adding the washing buffer.

HOW TO: The ASSIST PLUS pipetting robot transfers 500 µl of buffer RA3 from row C and D of the reservoir and instructs the user to switch on the vacuum pump. Apply a vacuum of -0.2 bar and release it after a 3 minute delay as indicated on the pipette.

5. Digestion of DNA

STEP: Avoid possible DNA contamination by directly applying rDNase onto the membrane.

HOW TO: The contaminating DNA that binds to the silica membrane needs to be removed. To achieve this, 95 µl of rDNase reaction mixture is transferred by ASSIST PLUS from row E of the reservoir directly above the silica membrane of each well. A 15 minute delay is preset in the program as an incubation period.

Tip:

- In this step, it is crucial to cover the silica membrane completely, so that the rDNase can come into contact with the entire silica membrane. This is achieved by optimizing the dispense speed and tip travel.

6. Washing

STEP: Wash the silica membrane in three steps.

HOW TO: The ASSIST PLUS pipetting robot adds 500 µl of buffer RA2 to each well (**Figure 5**). Apply a vacuum of -0.2 bar for 1 minute. In the meantime, the pipette instructs the user to exchange reservoir 1 for reservoir 2. After this, release the vacuum. The ASSIST PLUS transfers 800 µl of buffer RA3 to each well for a second wash step. Using the same parameters as before, apply the vacuum, release it, and allow the pipette to transfer 500 µl of buffer RA4 to each well for the third wash step. Apply the same vacuum as before for 1 minute, then release it.

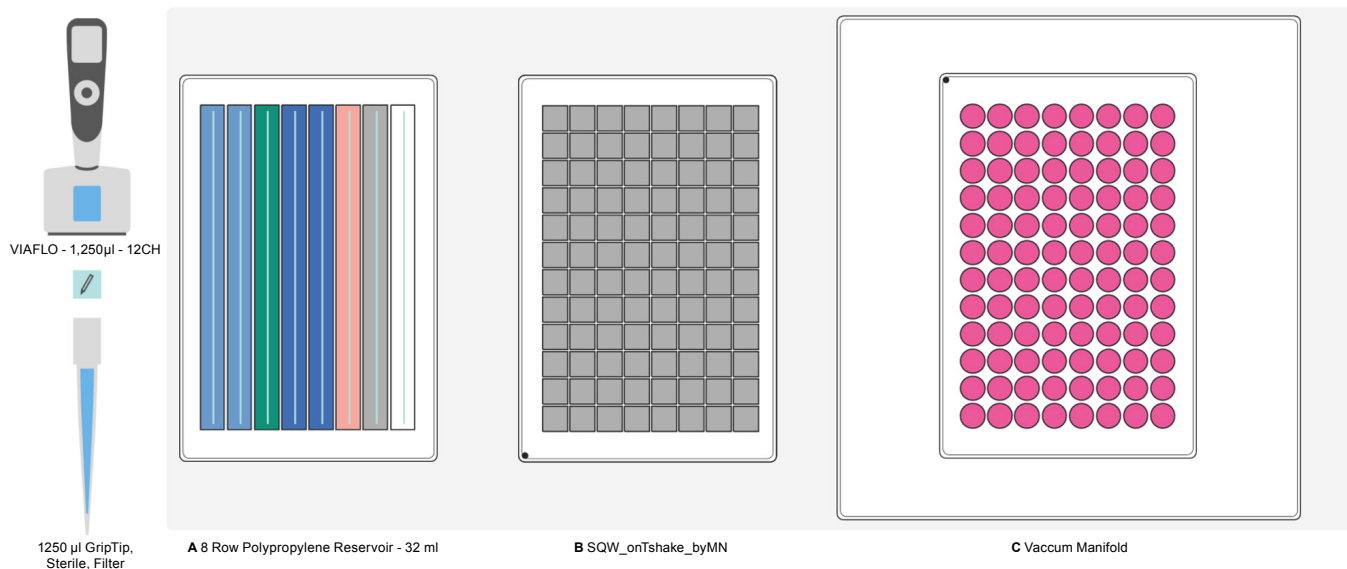


Figure 5: Deck set-up for the second and third wash steps. The 8 row reagent reservoir in **Position A** is filled with buffer RA3 in rows A to C, with buffer RA4 in rows D and E and with elution buffer in row F. In **Position C**, the wells in pink indicate the presence of RNA samples bound onto the silica membrane of the NucleoSpin 96 RNA binding plate.

7. Drying

STEP: Dry the RNA binding plate.

HOW TO: Following the final wash step with buffer RA4, remove the RNA binding plate from the vacuum manifold. Tap the plate on a clean paper towel to remove the residual ethanol-containing wash buffer at the bottom. Remove the wash plate and waste plate from the vacuum manifold. Set the vacuum to -0.6 bar. Insert the binding plate into the manifold lid, close the manifold and run the vacuum for 12 minutes in order to thoroughly dry the plate. Press RUN to activate the timer. An audible beep will occur at the end of the incubation period. Release the vacuum at this point.

8. Elution

STEP: Elute the RNA.

HOW TO: Remove the lid of the vacuum manifold and insert the U-bottom elution plate. Replace the lid of the manifold with the binding plate (**Figure 6**). Set the vacuum to -0.5 bar. Press RUN to activate the automated addition of elution buffer to the binding plate. Follow the prompt on the screen to incubate the plate for 3 minutes, followed by application of vacuum to release the RNA from the binding plate onto the elution plate.

After elution, discard the binding plate and take out the elution plate. The elution plate can either be directly used for downstream applications, or stored at -70 °C.

Tip:

- The elution buffer volume may be customized to meet the needs of individual users.

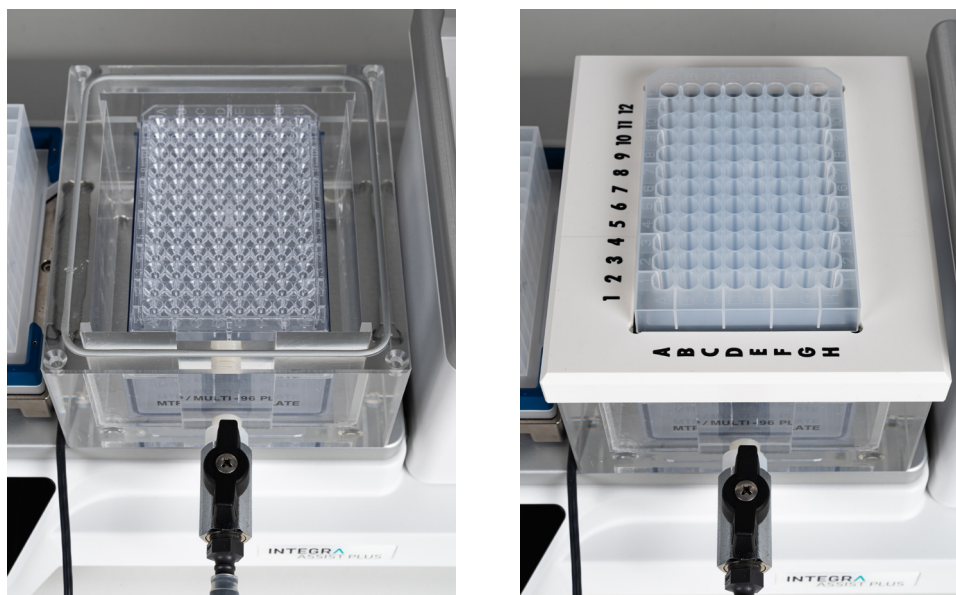


Figure 6: Vacuum manifold set-up for the elution step. The U-bottom elution plate is inserted onto the 'MTP/MULTI-96 PLATE' spacers inside the manifold base. The NucleoSpin 96 RNA Binding Plate with the RNA samples is placed on top of the manifold.

Results

To evaluate the above-described protocol, RNA was extracted from the same samples using both the ASSIST PLUS pipetting robot and a handheld pipette (**Figure 7**). Overall, the RNA yield was found to be higher with the ASSIST PLUS than if processed manually. RNA purity with the ASSIST PLUS was comparable to the manual process but, the standard deviation was smaller.

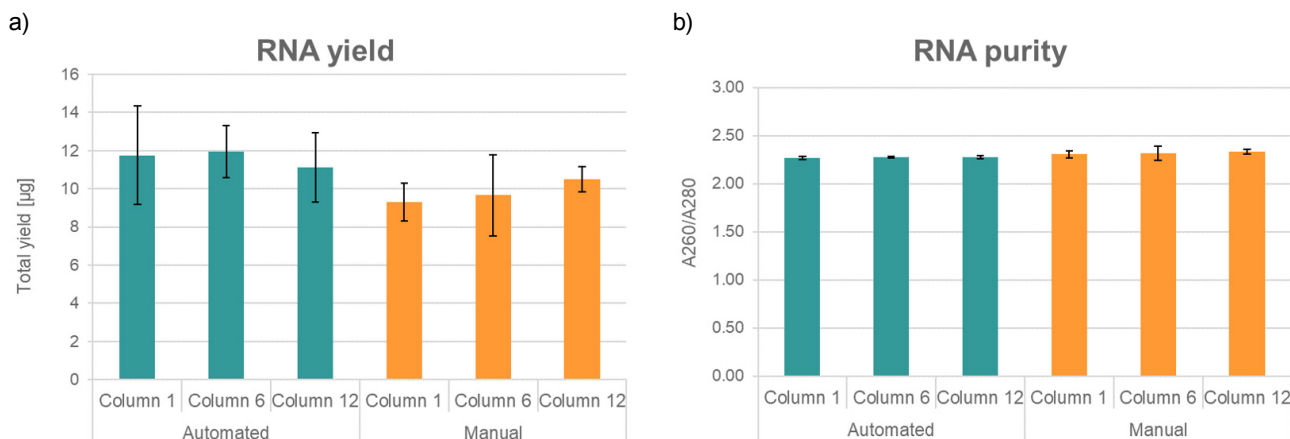


Figure 7: High quality RNA obtained from tissue samples (approx. 10 mg per sample). a) yield and b) purity were determined by UV spectroscopy. N = 8 for each column.

The high RIN scores of the eight HeLa cell replicates (**Figure 8**) indicate an excellent RNA integrity and high consistency.

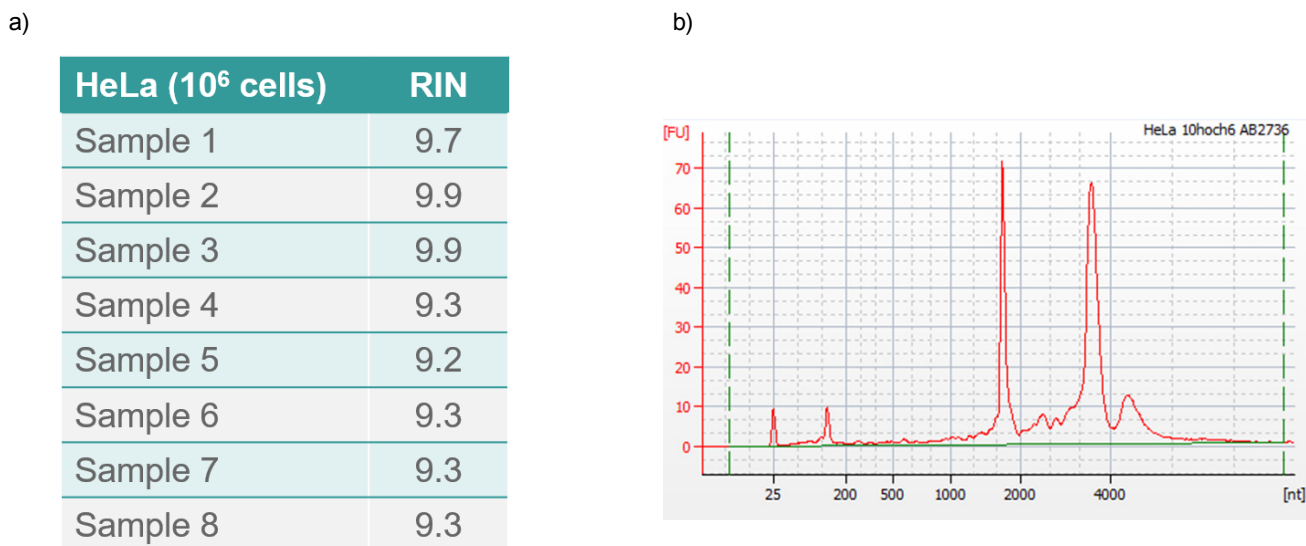


Figure 8: a) High RNA integrity number (RIN) obtained from 8 replicates of HeLa cells (10⁶), b) electropherogram showing one example of RIN of HeLa cells (10⁶).

Remark

Programs can be adapted to a different number of samples at any time, giving laboratories total flexibility to meet current and future demands.

Conclusion

- Automation of the MACHEREY-NAGEL NucleoSpin 96 RNA kit can be easily achieved on the ASSIST PLUS pipetting robot using a 12 channel VIAFLO electronic pipette.
- The MACHEREY-NAGEL NucleoSpin 96 RNA Kit yields high quality RNA, and automation of the protocol eliminates the possibility of inconsistent pipetting or user-induced errors. The ASSIST PLUS pipetting robot never gets tired and, consequently, eliminates variability in results obtained by different operators.
- Users may customize any step in the program using INTEGRA's free VIALAB software, or with support from INTEGRA's representatives.
- Thanks to its compact footprint, the ASSIST PLUS pipetting robot can be easily placed in laminar flow cabinets to protect samples from any contamination.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA	4634	VIAFLO 12 channel 1250 µl electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo
INTEGRA	4221	Pipette Communication Module for INTEGRA electronic pipettes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA	6445	1250 µl Sterile, Filter GripTips	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA	6372	8 Row Reagent Reservoirs, Pyramid Bottom, Partitioned (32 ml/row) Pre-Sterilized, 25 units, Polypropylene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA	128152	INHECO Teleshake SBS Adapter	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INHECO	3800047 3800048	Teleshake 230 V (EU) Teleshake 115 V (US)	https://www.inheco.com/teleshake.html
MACHEREY-NAGEL	740709.2 740709.4 740709.24	NucleoSpin 96 RNA Kit	https://www.mn-net.com/ch/nucleospin-96-rna-96-well-kit-for-rna-purification-740709.4?c=4244
MACHEREY-NAGEL	740681	NucleoVac 96 Vacuum Manifold	https://www.mn-net.com/ch/nucleovac-96-vacuum-manifold-740681
VACUUBRAND	MZ 2 NT	Vacuum Pump	

Appendix: Performing the initial deck set-up

Check that the cable of the Teleshake (**Position B**) is not interfering with the movement of the ASSIST PLUS tower. Also, make sure that the outlet of the vacuum manifold (**Position C**) is positioned towards the user, so that the tower of the ASSIST PLUS can move freely along the X axis (**Figure 1**).

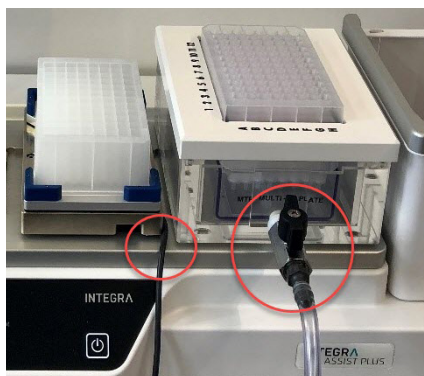
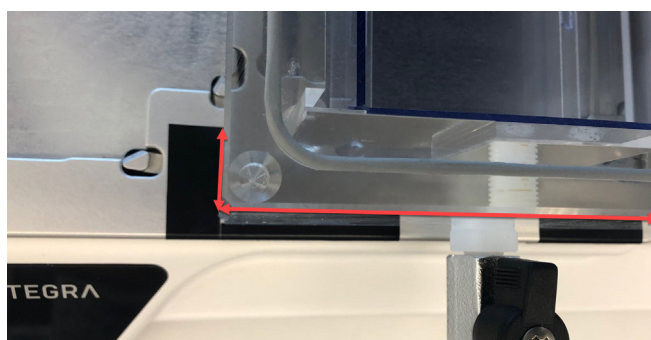
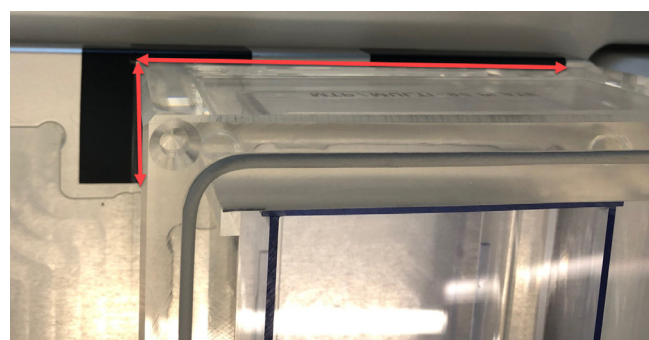


Figure 1: Positioning of the Teleshake cable and the outlet of the vacuum manifold.

Place the vacuum manifold on the ASSIST PLUS deck next to the waste bin. After adjusting the position of the manifold the first time, it's recommended to mark its position on the deck (see example in **Figure 2**). Thereafter, simply align the vacuum manifold with the marks placed on the ASSIST PLUS.



Front



Back

Figure 2: Alignment of the manifold on the deck in **Position C**. Adding marks on the deck helps to reposition the manifold whenever needed.

To check the position of the well plate on top of the vacuum manifold, attach the tips to the pipette manually. Use the touch panel keys to move the pipetting arm of the ASSIST PLUS and to control the tip position. First, select 'ASSIST PLUS' from the main menu of the pipette followed by 'VIALAB Programs' and 'MN RNA CELL'. After this, go to 'Height Adjust', select '7 Transfer' and then choose 'Height 1/1' under 'Target' using the left arrow. Confirm selection by pressing the start key on the ASSIST PLUS. The ASSIST PLUS then moves to the chosen wells. Check the position of the vacuum manifold. The pipette tips should always be in the middle of the wells. If necessary, manually adjust the position of the vacuum manifold on the deck. Press the 'back' button on the pipette to exit the 'Height adjust' menu and discard the tips manually. Continue with the protocol set-up.

INTEGRA Biosciences AG
7205 Zizers, Switzerland
T +41 81 286 95 30
F +41 81 286 95 33
info@integra-biosciences.com

INTEGRA Biosciences Corp.
Hudson, NH 03051, USA
T +1 603 578 5800
F +1 603 577 5529
info-us@integra-biosciences.com

INTEGRA Biosciences Deutschland GmbH
35444 Biebertal, Deutschland
T +49 6409 81 999 15
F +49 6409 81 999 68
info-de@integra-biosciences.com

INTEGRA Biosciences SAS
95062 Cergy-Pontoise Cedex 1, France
T +33 (0)1 34 30 76 76
F +33 (0)1 34 30 76 79
info-fr@integra-biosciences.com

INTEGRA Biosciences Ltd.
Egham, Surrey TW20 9EY, UK
info-uk@integra-biosciences.com