MN RNA TISSUE Report

Program Name (on pipette)
MN RNA Tissue

INTEGRA VIALAB

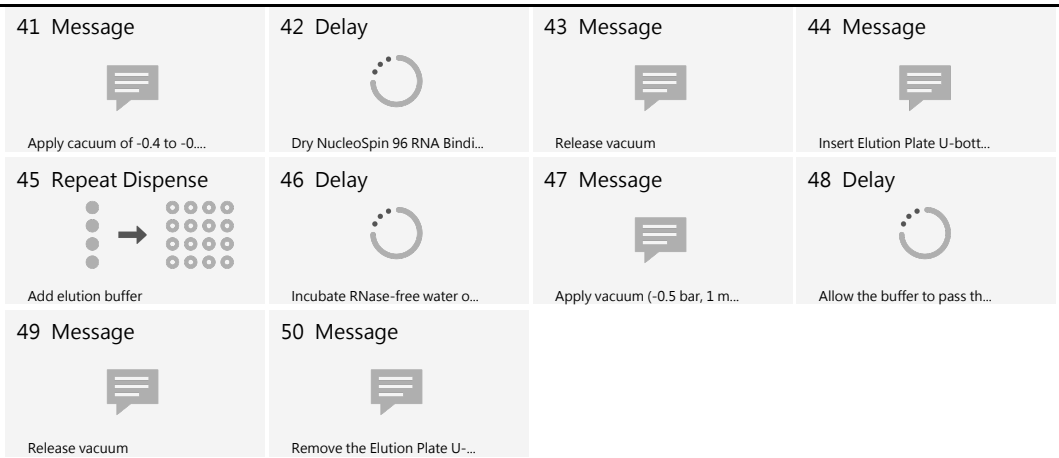
User Credentials

Name: eva.meszaros
Date: 28. Jan. 2022

Overview Method

VIAFLO - 1,250µl - 12CH

1 Initial Volumes Fill Reservoir 1...	2 Repeat Dispense Add Buffer RA4	3 Transfer Mix and transfer crude lysa...	4 Message Apply vacuum (-0.2 bar, 1 m...
5 Delay Wait until the crude lysate...	6 Message Release vacuum	7 Volume Change	8 Repeat Dispense Add Buffer RA3 to rows 1-4
9 Repeat Dispense Add Buffer RA3 to rows 5-8	10 Message Apply vacuum (-0.2 bar, 1 m...	11 Delay Allow the buffer to pass th...	12 Message Release vacuum
13 Volume Change	14 Repeat Dispense Add rDNase	15 Delay Digest DNA for 15 min	16 Volume Change
17 Repeat Dispense Add Buffer RA2 to rows 1-4,...	18 Repeat Dispense Add Buffer RA2 to rows 5-8,...	19 Message Apply vacuum (-0.2 bar; 1 m...	20 Delay
21 Message Exchange Buffer Rack...	22 Message Release vacuum	23 Volume Change	24 Volume Change
25 Repeat Dispense Add Buffer RA3 to rows 1-3,...	26 Repeat Dispense Add Buffer RA3 to rows 4-6,...	27 Repeat Dispense Add Buffer RA3 to rows 7-8,...	28 Message Apply vacuum (-0.2 bar, 1 m...
29 Delay Allow the buffer to pass th...	30 Message Release vacuum	31 Volume Change	32 Repeat Dispense Add Bueer RA4 to rows 1-4, ...
33 Repeat Dispense Add Buffer RA4 to rows 5-8,...	34 Message Apply vacuum (-0,2 bar, 1 m...	35 Delay Allow the buffer to pass th...	36 Message Release vacuum
37 Volume Change	38 Message Remove MN Wash Plate and Wa...	39 Message Remove any residual wash bu...	40 Message Close the manifold base wit...



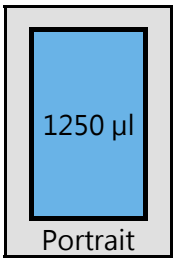
Total Time: 57 min 23 sec

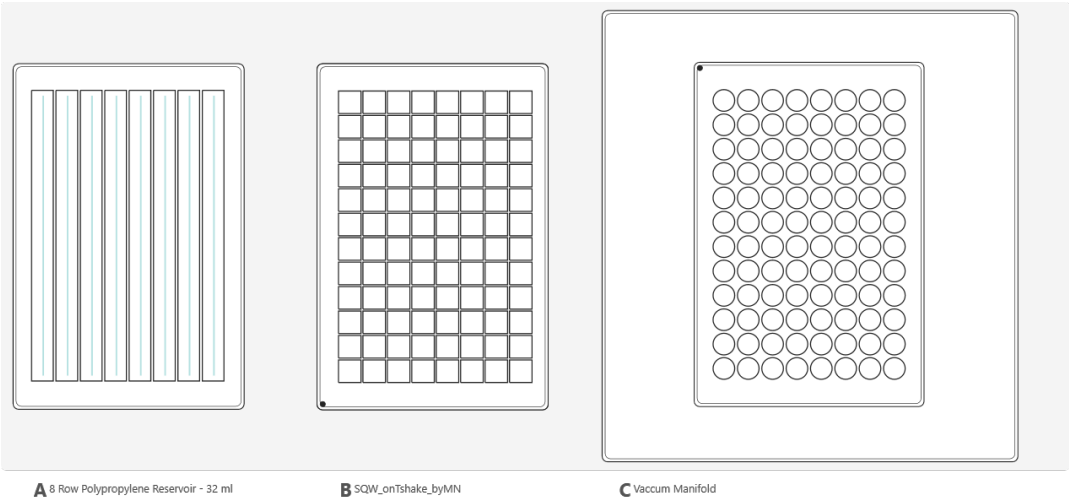
Total Tip Consumption: 180

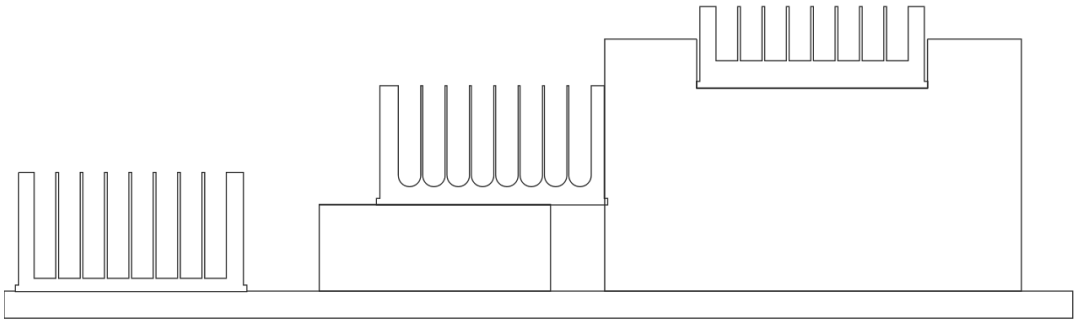
Description

This Program is written according to the NucleoSpin 96 RNA Kit user manual for optimal usage of the Kit with the ASSIST PLUS pipetting robot, and Teleshake.

Deck Layout







Pipette & Deck

Labware	Name	Manufacturer	Part Number
Pipette	VIAFLO 1,250µl 12 channels	INTEGRA	4634
Pipette Tip	1250 µl GripTip, Sterile, Filter	INTEGRA	6445
Deck	3 Position Deck_Macherey Nagel	INTEGRA	

Deck Labware

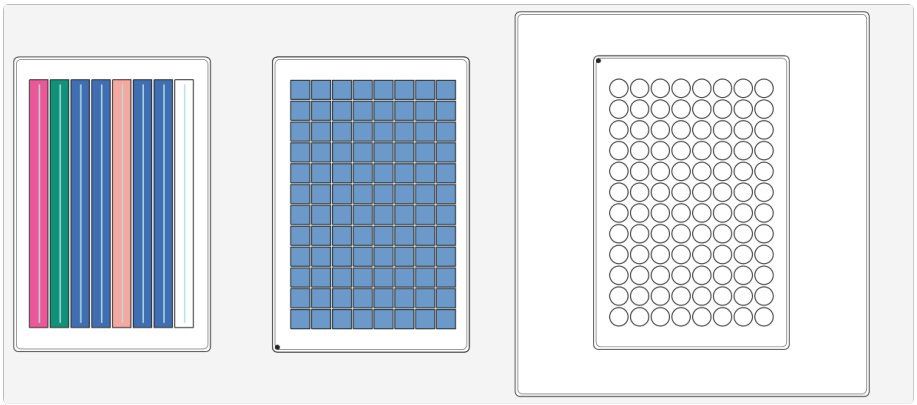
Deck Position	Labware	Name	Manufacturer	Part Number	Description
A	Reservoir	8 Row Polypropylene Reservoir - 32 ml	INTEGRA	6371, 6372	
B	COMBI System	SQW_onTshake_byMN	Macherey Nagel	740481	- shaking device - including F-bottom adapter
	B1	COMBISystem_96 Deepwell U-bottom_Culture Plate_Macherey Nagel	Ritter Medical (Riplate)	740488,740488.24	Culture plate provided in Plasmid DNA Purification kit Macherey Nagel PN 740625.1
C	COMBI System	Vaccum Manifold			
	C1	Filter plate macherey nagel	Macherey Nagel		Automatically rotated
D	Waste				

Method

Step	Description
1 Initial Volum...	

Description:
Fill Reservoir 1

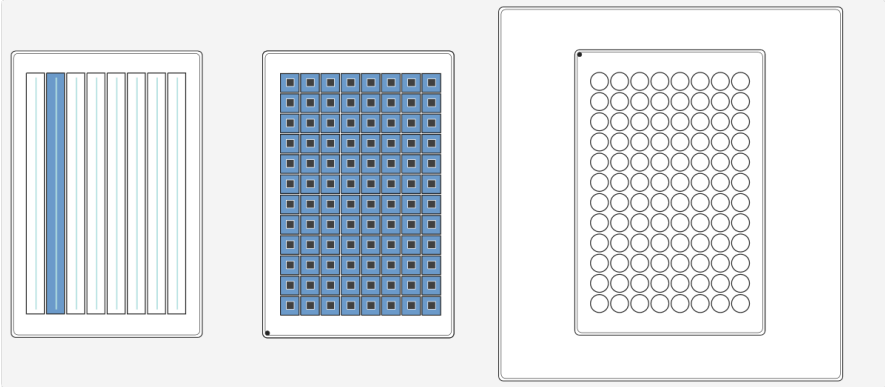
Row B: 30 ml buffer RA4
Row C and D: 25 ml buffer RA3
Row E: 10 ml rDNase
Row F and G: 25 ml RA2



2 Repeat Dispen...

Time:
1 min 17 sec
Used Tips:
12

Description:
Add Buffer RA4



Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A2	32 mm	B	B1 A12-A1	65 mm	300
2	A	A2	32 mm	B	B1 B12-B1	65 mm	300
3	A	A2	32 mm	B	B1 C12-C1	65 mm	300
4	A	A2	32 mm	B	B1 D12-D1	65 mm	300
5	A	A2	32 mm	B	B1 E12-E1	65 mm	300
6	A	A2	32 mm	B	B1 F12-F1	65 mm	300
7	A	A2	32 mm	B	B1 G12-G1	65 mm	300
8	A	A2	32 mm	B	B1 H12-H1	65 mm	300

Pipetting settings

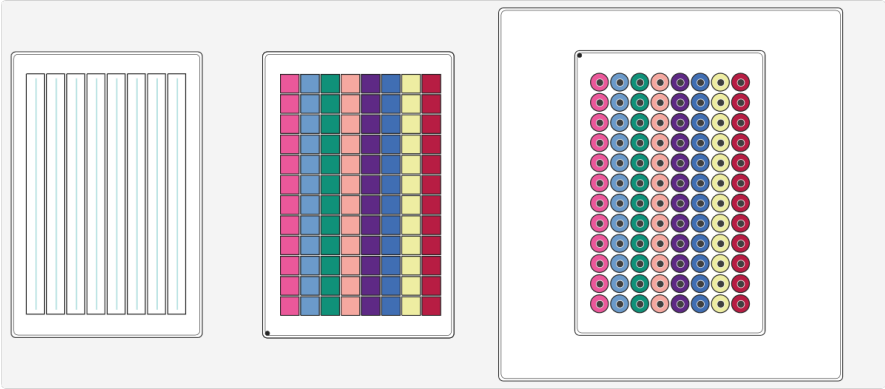
Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** B1: 9 mm
Volumes	Volume Post-Dispense Post-Dispense Location Reuse Post-Dispense Dispense Type Air Gap	Fix 30 µl Source No Multi 30 µl

Step	Description		
	Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	6 0 6 0 No
	Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** B1: Fix No B1: 2 mm
	Tip Change	Tip Change	After step complete
	Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
	Tip Touch	Tip Touch	No

3 Transfer

Time:
7 min 45 sec
Used Tips:
96

Description:
Mix and transfer crude lysates to the NucleoSpin RNA Binding Plate

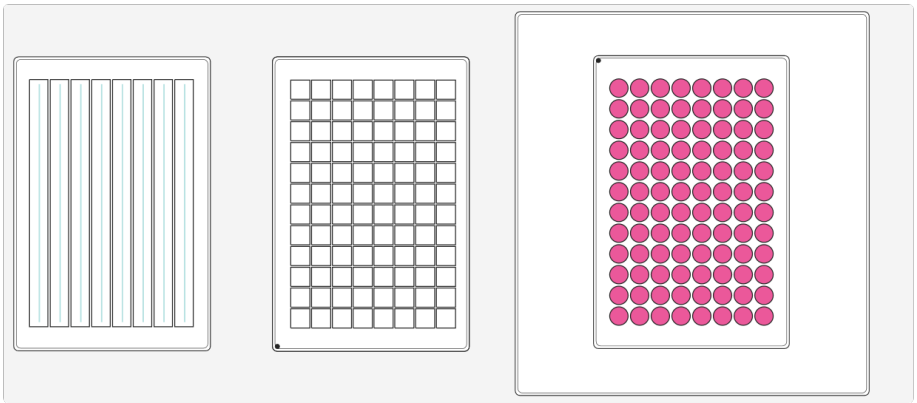
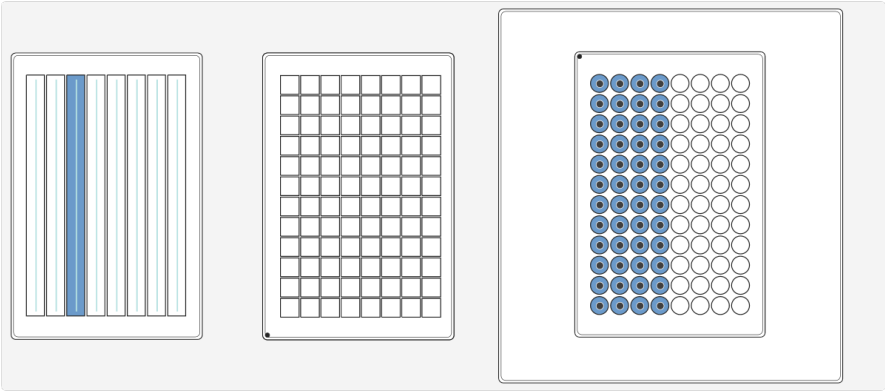


Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	B	B1 A12-A1	43 mm	C	C1 A1-L1	105 mm	600
2	B	B1 B12-B1	43 mm	C	C1 A2-L2	105 mm	600
3	B	B1 C12-C1	43 mm	C	C1 A3-L3	105 mm	600
4	B	B1 D12-D1	43 mm	C	C1 A4-L4	105 mm	600
5	B	B1 E12-E1	43 mm	C	C1 A5-L5	105 mm	600
6	B	B1 F12-F1	43 mm	C	C1 A6-L6	105 mm	600
7	B	B1 G12-G1	43 mm	C	C1 A7-L7	105 mm	600
8	B	B1 H12-H1	43 mm	C	C1 A8-L8	105 mm	600

Pipetting settings

Step	Description		
	Tab	**Parameter**	**Set value**
	Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** B1: 9 mm **Target:** C1: 9 mm
	Volumes	Volume	Fix
	Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	5 0 6 0 No
	Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** B1: Fix Yes B1: 0.1 mm **Target:** C1: Variable No C1: 2 mm
	Tip Change	Tip Change	After each transfer
	Mix	**Source:** Mixing Mix Volume Mix Cycles Mix Speed Tip Travel Start Height **Target:** Mixing	**Source:** Yes B1: 500 µl 12 6 Yes B1: 43 mm **Target:** No
	Tip Touch	Tip Touch	No
4 Message **Description:** Apply vacuum (-0.2 bar, 1 min) If necessary, press down the NucleoSpin RNA Binding Plate slightly until flow through starts	Pipetting settings		
	Tab	**Parameter**	**Set value**
	Message	Message Line 1 Message Line 2 Message Line 3	Apply vacuum -200 mbar 1 min

Step	Description
5 Delay **Description:** Wait until the crude lysate has passed the NucleoSpin RNA Bindng Plate	Pipetting settings **Tab**: **Delay** **Parameter**: Delay Time Skip Delay by RUN **Set value**: 60 Yes
6 Message **Description:** Release vacuum	Pipetting settings **Tab**: **Message** **Parameter**: Message Line 1 Message Line 2 Message Line 3 **Set value**: Release vacuum -
7 Volume Change	
8 Repeat Dispen... **Time:** 1 min 7 sec **Used Tips:** 12 **Description:** Add Buffer RA3 to rows 1-4	 Summary individual transfers

Step	Description

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A3	31.9 mm	C	C1 A1-L1	110 mm	500
2	A	A3	31.9 mm	C	C1 A2-L2	110 mm	500
3	A	A3	31.9 mm	C	C1 A3-L3	110 mm	500
4	A	A3	31.9 mm	C	C1 A4-L4	110 mm	500

Pipetting settings

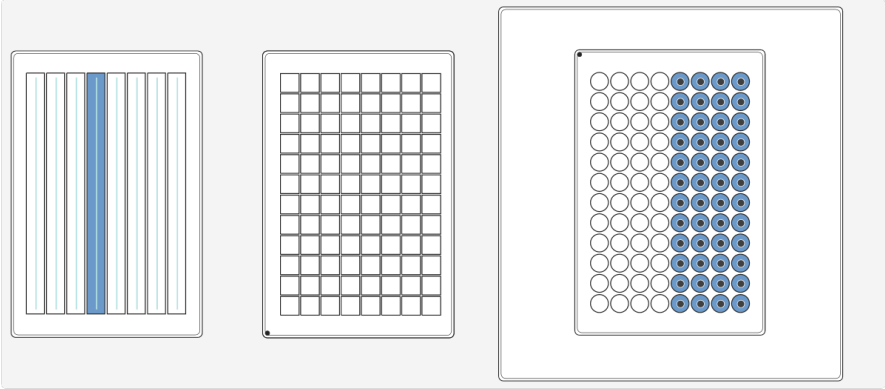
Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 50 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	6 0 2 1 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	Continue to next step without change
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

9 Repeat Dispen...

Time:
1 min 6 sec
Used Tips:
0

Description:
Add Buffer RA3 to rows 5-8

Step	Description



Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A4	31.9 mm	C	C1 A5-L5	110 mm	500
2	A	A4	31.9 mm	C	C1 A6-L6	110 mm	500
3	A	A4	31.9 mm	C	C1 A7-L7	110 mm	500
4	A	A4	31.9 mm	C	C1 A8-L8	110 mm	500

Pipetting settings

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 50 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	6 0 2 1 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	After step complete
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No

Step	Description		
	Tip Touch	Tip Touch	No

10 Message

Description:
Apply vacuum (-0.2 bar, 1 min)
If necessary, press down the NucleoSpin RNA Binding Plate slightly until flow through starts

Pipetting settings

Tab	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Apply vacuum -200 mbar 3 min

11 Delay

Description:
Allow the buffer to pass the wells

Pipetting settings

Tab	Parameter	Set value
Delay	Delay Time Skip Delay by RUN	180 Yes

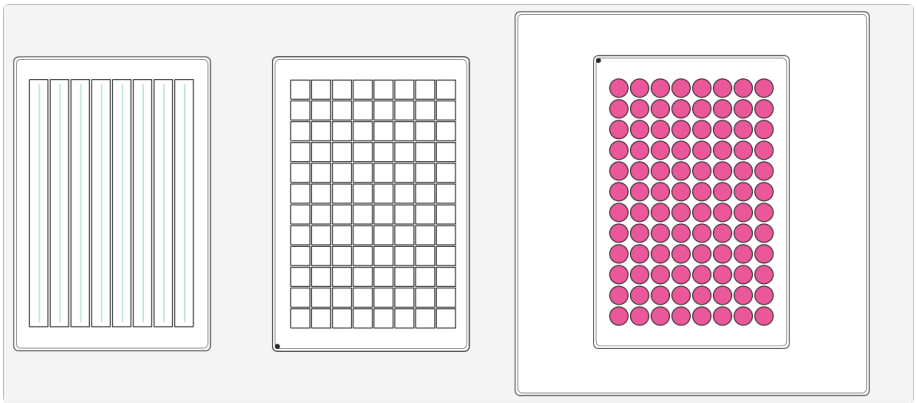
12 Message

Description:
Release vacuum

Pipetting settings

Tab	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Release vacuum -

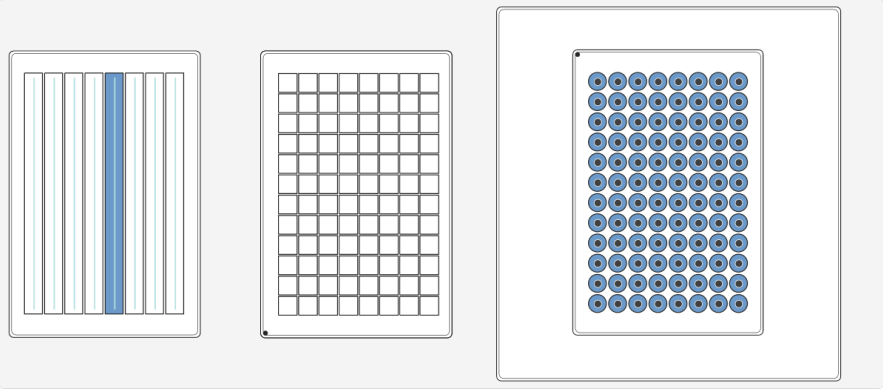
13 Volume Change



Step	Description
14 Repeat Dispen...	

Time:
1 min 1 sec
Used Tips:
12

Description:
Add rDNase



Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A5	10 mm	C	C1 A1-L1	100 mm	95
2	A	A5	10 mm	C	C1 A2-L2	100 mm	95
3	A	A5	10 mm	C	C1 A3-L3	100 mm	95
4	A	A5	10 mm	C	C1 A4-L4	100 mm	95
5	A	A5	10 mm	C	C1 A5-L5	100 mm	95
6	A	A5	10 mm	C	C1 A6-L6	100 mm	95
7	A	A5	10 mm	C	C1 A7-L7	100 mm	95
8	A	A5	10 mm	C	C1 A8-L8	100 mm	95

Pipetting settings

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 40 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	6 0 3 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** C1: Fix Yes C1: 2 mm
Tip Change	Tip Change	After step complete

Step	Description		
	Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
	Tip Touch	Tip Touch	No

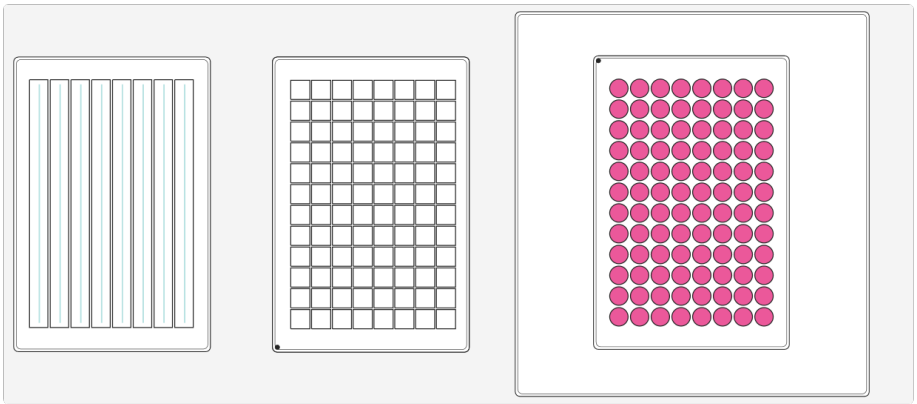
15 Delay

Description:
Digest DNA for 15 min

Pipetting settings

Tab	Parameter	Set value
Delay	Delay Time Skip Delay by RUN	900 Yes

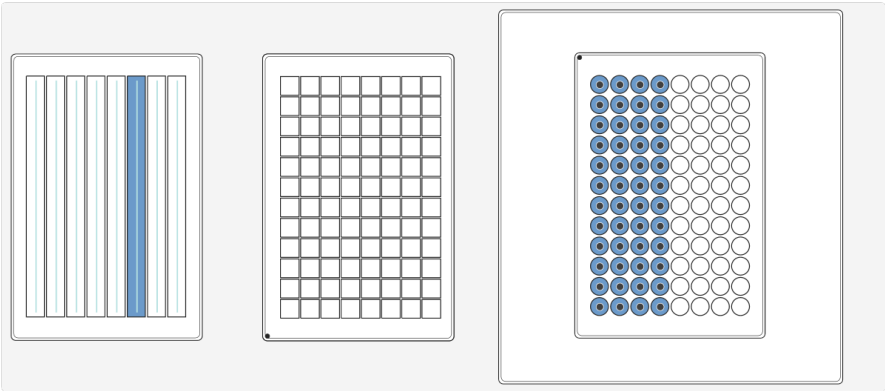
16 Volume Change



17 Repeat Dispen...

Time:
0 min 49 sec
Used Tips:
12

Description:
Add Buffer RA2 to rows 1-4, first wash



Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A6	31.9 mm	C	C1 A1-L1	110 mm	500
2	A	A6	31.9 mm	C	C1 A2-L2	110 mm	500

Step	Description

3	A	A6	31.9 mm	C	C1 A3-L3	110 mm	500
4	A	A6	31.9 mm	C	C1 A4-L4	110 mm	500

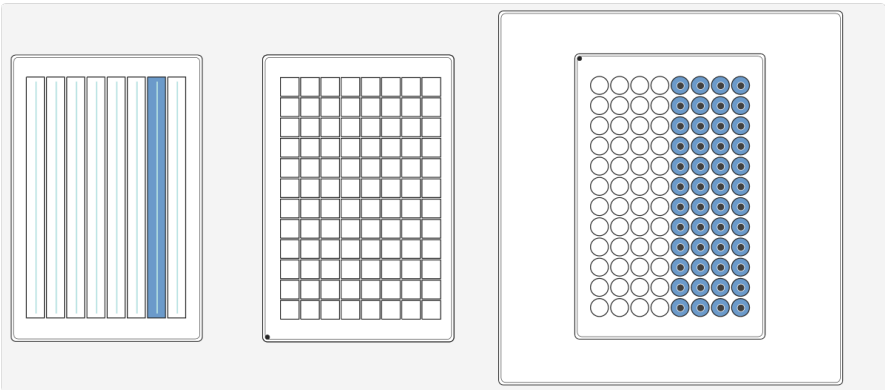
Pipetting settings

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 50 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	5 0 4 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 2 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	Continue to next step without change
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

18 Repeat Dispen...

Time:
0 min 46 sec
Used Tips:
0

Description:
Add Buffer RA2 to rows 5-8, first wash



Summary individual transfers

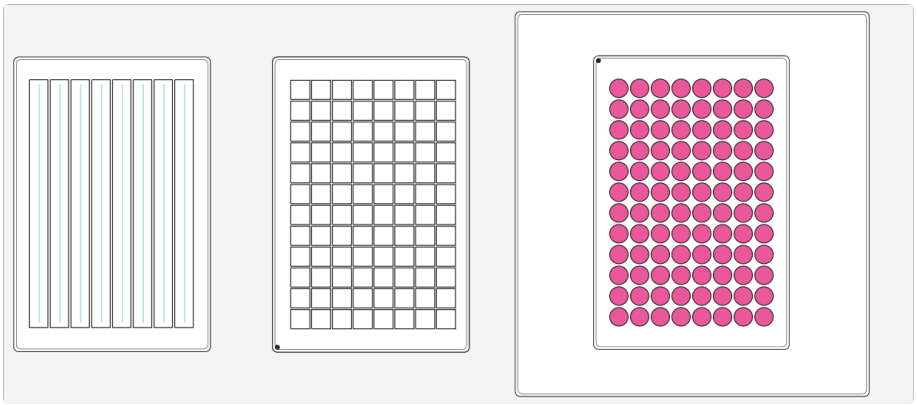
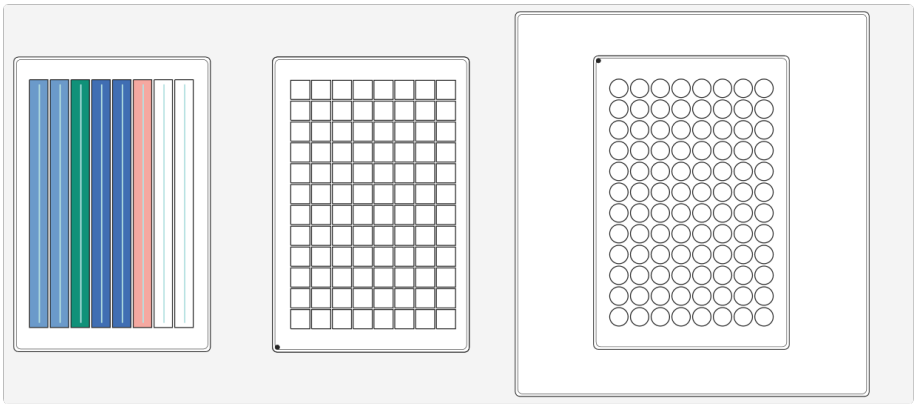
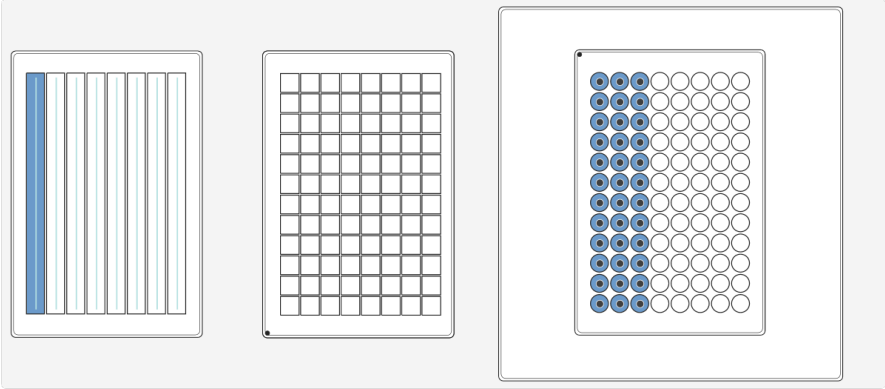
Step	Description

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A7	31.9 mm	C	C1 A5-L5	110 mm	500
2	A	A7	31.9 mm	C	C1 A6-L6	110 mm	500
3	A	A7	31.9 mm	C	C1 A7-L7	110 mm	500
4	A	A7	31.9 mm	C	C1 A8-L8	110 mm	500

Pipetting settings

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 50 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	6 0 4 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 2 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	After step complete
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

Step	Description
19 Message **Description:** Apply vacuum (-0.2 bar; 1 min) If necessary, press down the NucleoSpin RNA Binding Plate slightly until flow through starts	Pipetting settings Tab: **Message** Parameter: Message Line 1 / Message Line 2 / Message Line 3 Set value: Apply vacuum / -200 mbar / 1 min
20 Delay	Pipetting settings Tab: **Delay** Parameter: Delay Time / Skip Delay by RUN Set value: 60 / Yes
21 Message **Description:** Exchange Buffer Rack 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	Pipetting settings Tab: **Message** Parameter: Message Line 1 / Message Line 2 / Message Line 3 Set value: Exchange / Buffer Rack / -
22 Message **Description:** Release vacuum	Pipetting settings Tab: **Message** Parameter: Message Line 1 / Message Line 2 / Message Line 3 Set value: Release / vacuum / -

Step	Description
23 Volume Change	
24 Volume Change	
25 Repeat Dispen... **Time:** 0 min 53 sec **Used Tips:** 12 **Description:** Add Buffer RA3 to rows 1-3, second wash	

Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A1	31.9 mm	C	C1 A1-L1	110 mm	800
2	A	A1	31.9 mm	C	C1 A2-L2	110 mm	800
3	A	A1	31.9 mm	C	C1 A3-L3	110 mm	800

Pipetting settings

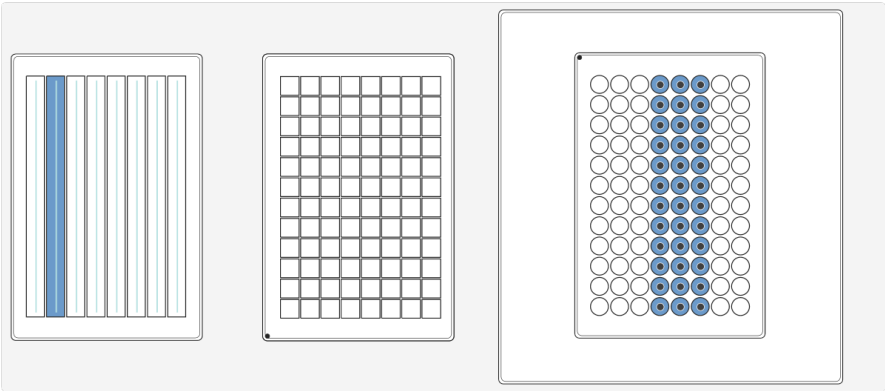
Step	Description

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Single 40 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	5 0 4 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	Continue to next step without change
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

26 Repeat Dispen...

Time:
0 min 45 sec
Used Tips:
0

Description:
Add Buffer RA3 to rows 4-6, second wash



Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A2	31.9 mm	C	C1 A4-L4	110 mm	800
2	A	A2	31.9 mm	C	C1 A5-L5	110 mm	800

Step	Description

3	A	A2	31.9 mm	C	C1 A6-L6	110 mm	800

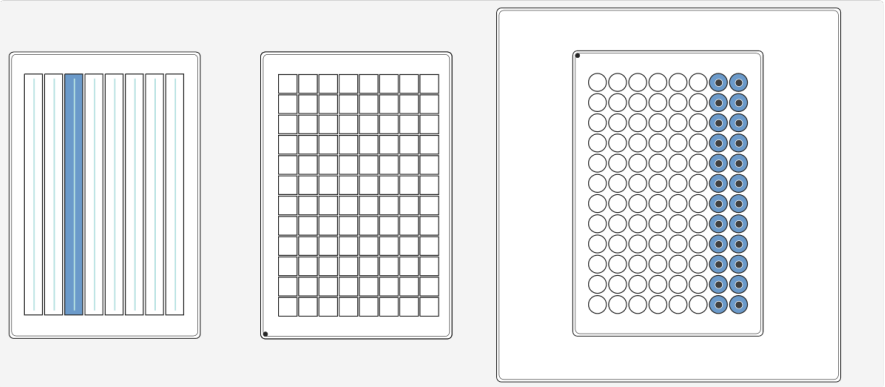
Pipetting settings

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Single 40 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	5 0 4 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	Continue to next step without change
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

27 Repeat Dispen...

Time:
0 min 37 sec
Used Tips:
0

Description:
Add Buffer RA3 to rows 7-8, second wash



Summary individual transfers

Step	Description

Step	Source			Target			Volume [µl]
	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	
1	A	A3	17 mm	C	C1 A7-L7	110 mm	800
2	A	A3	17 mm	C	C1 A8-L8	110 mm	800

Pipetting settings

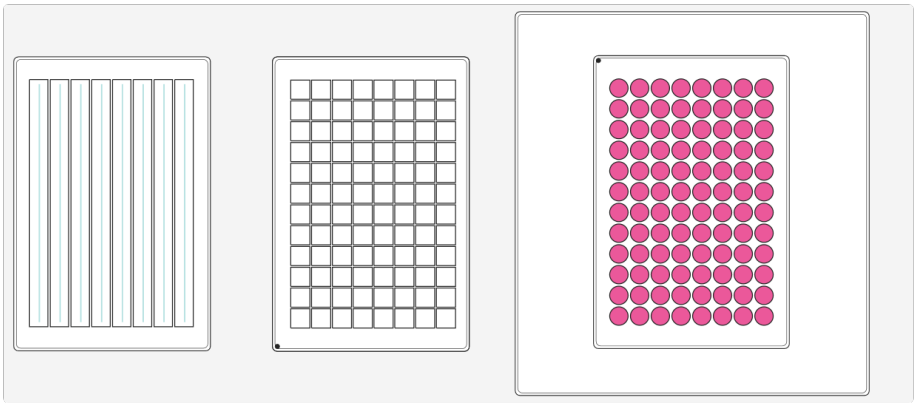
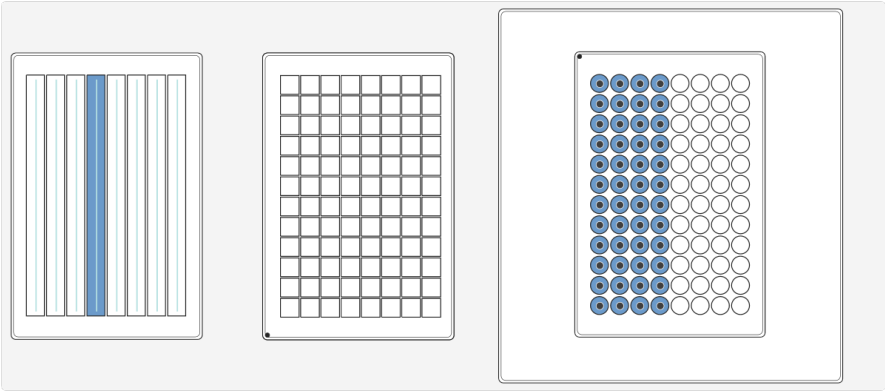
Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Single 40 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	5 0 4 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	After step complete
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

28 Message

Description:
Apply vacuum (-0.2 bar, 1 min)
If necessary, press down the NucleoSpin RNA Binding Plate slightly until flow through starts

Pipetting settings

Tab	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Apply vacuum -200 mbar 1 min

Step	Description
29 Delay **Description:** Allow the buffer to pass the wells	Pipetting settings Tab: **Delay** Parameter: Delay Time Set value: 60 Parameter: Skip Delay by RUN Set value: Yes
30 Message **Description:** Release vacuum	Pipetting settings Tab: **Message** Parameter: Message Line 1 Set value: Release Parameter: Message Line 2 Set value: vacuum Parameter: Message Line 3 Set value: -
31 Volume Change	
32 Repeat Dispen... **Time:** 0 min 45 sec **Used Tips:** 12 **Description:** Add Bueer RA4 to rows 1-4, third wash	 Summary individual transfers

Step	Description

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A4	31.9 mm	C	C1 A1-L1	110 mm	500
2	A	A4	31.9 mm	C	C1 A2-L2	110 mm	500
3	A	A4	31.9 mm	C	C1 A3-L3	110 mm	500
4	A	A4	31.9 mm	C	C1 A4-L4	110 mm	500

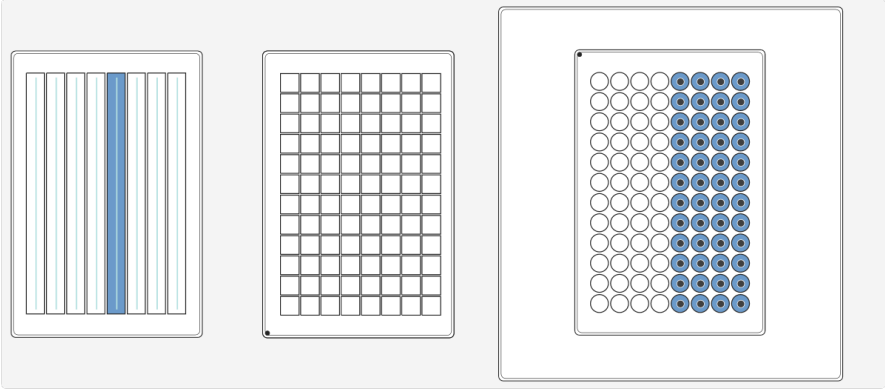
Pipetting settings

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 50 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	8 0 4 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 2 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	Continue to next step without change
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

33 Repeat Dispen...

Time:
0 min 44 sec
Used Tips:
0

Step	Description
Description: Add Buffer RA4 to rows 5-8, third wash	



Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A5	31.9 mm	C	C1 A5-L5	110 mm	500
2	A	A5	31.9 mm	C	C1 A6-L6	110 mm	500
3	A	A5	31.9 mm	C	C1 A7-L7	110 mm	500
4	A	A5	31.9 mm	C	C1 A8-L8	110 mm	500

Pipetting settings

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 50 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	8 0 4 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 2 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	After step complete
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No

Step	Description		
	Tip Touch	Tip Touch	No

34 Message

Description:
Apply vacuum (-0,2 bar, 1 min)
If necessary, press down the NucleoSpin RNA Binding Plate slightly until flow through starts

Pipetting settings

Tab	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Apply vacuum -200 mbar 1 min

35 Delay

Description:
Allow the buffer to pass the wells

Pipetting settings

Tab	Parameter	Set value
Delay	Delay Time Skip Delay by RUN	60 Yes

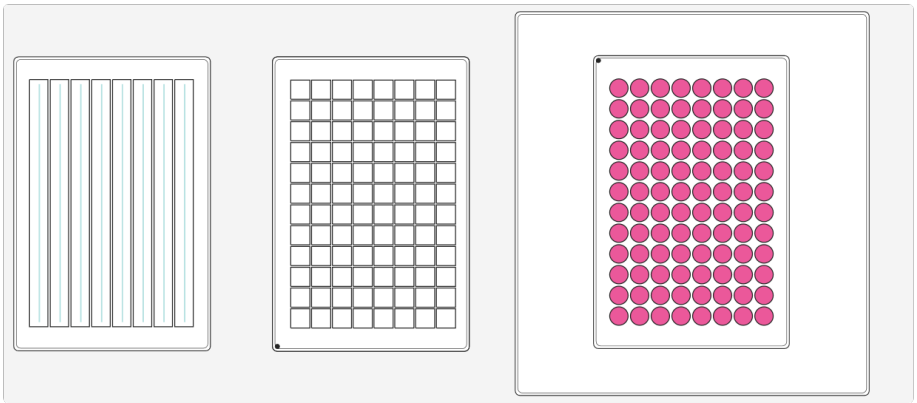
36 Message

Description:
Release vacuum

Pipetting settings

Tab	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Release vacuum -

37 Volume Change



Step	Description
38 Message **Description:** Remove MN Wash Plate and Waste Container from the inside of the vacuum manifold Empty the waste container	Pipetting settings Tab: **Message** Parameter: Message Line 1 / Message Line 2 / Message Line 3 Set value: Remove MN Wash Plate and Waste
39 Message **Description:** Remove any residual wash buffer from the NucleoSpin RNA Binding Plate If necessary, tap the outlets of the plate onto a clean paper sheet (supplied with the MN Wash Plate) or soft tissue until no drops come out	Pipetting settings Tab: **Message** Parameter: Message Line 1 / Message Line 2 / Message Line 3 Set value: Remove drops from Binding Plate
40 Message **Description:** Close the manifold base with the manifold lid Place the NucleoSpin RNA Binding Plate on top of the manifold. Build up the vacuum with the valve closed Once the maximum vacuum (-0.6 bar) is achieved, open the valve and apply vacuum for at least 10 min to dry the membrane completely This step is necessary to eliminate traces of ethanol	Pipetting settings Tab: **Message** Parameter: Message Line 1 / Message Line 2 / Message Line 3 Set value: Assemble vac manifold for drying

Step	Description
41 Message **Description:** Apply cacuum of -0.4 to -0.6 bar for at least 10-15 min to dry the membrane completely Run vacuum pump continuously Typically, the adjusted vacuum is not reached at this step Achieving and keeping a continuous air-flow in order to evaporate the remaining ethanol is of more importance than reaching the precise mentioned atmospheric pressure	Pipetting settings **Tab**: **Message** **Parameter**: Message Line 1, Message Line 2, Message Line 3 **Set value**: Vacuum, -600 mbar, 12 min
42 Delay **Description:** Dry NucleoSpin 96 RNA Binding Plate	Pipetting settings **Tab**: **Delay** **Parameter**: Delay Time, Skip Delay by RUN **Set value**: 720, Yes
43 Message **Description:** Release vacuum	Pipetting settings **Tab**: **Message** **Parameter**: Message Line 1, Message Line 2, Message Line 3 **Set value**: Release, vacuum, -

Step	Description
44 Message	

Description:
Insert Elution Plate U-bottom
Remove the manifold lid with the NucleoSpin RNA Binding Plate from the vacuum manifold
Insert the Elution Plate on the spacers 'MTP/MULTI-96 PLATE' inside the manifold base
Close the manifold base with the manifold lid
Place the NucleoSpin® RNA Binding Plate on top of the manifold

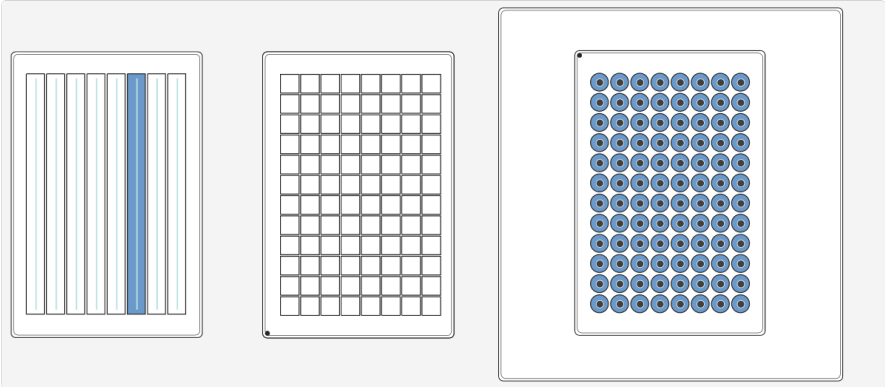
Pipetting settings

Tab	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Assemble vac manifold for elution

45 Repeat Dispen...

Time:
1 min 36 sec
Used Tips:
12

Description:
Add elution buffer



Summary individual transfers

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
1	A	A6	10 mm	C	C1 A1-L1	88 mm	100
2	A	A6	10 mm	C	C1 A2-L2	88 mm	100
3	A	A6	10 mm	C	C1 A3-L3	88 mm	100
4	A	A6	10 mm	C	C1 A4-L4	88 mm	100
5	A	A6	10 mm	C	C1 A5-L5	88 mm	100
6	A	A6	10 mm	C	C1 A6-L6	88 mm	100
7	A	A6	10 mm	C	C1 A7-L7	88 mm	100
8	A	A6	10 mm	C	C1 A8-L8	88 mm	100

Pipetting settings

Step	Description

Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type	Fix Single
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	6 0 9 0 No
Pipetting Height	**Source:** Heights Tip Travel Safety Bottom Offset **Target:** Heights Tip Travel Safety Bottom Offset	**Source:** Fix Yes 1.3 mm **Target:** C1: Fix No C1: 2 mm
Tip Change	Tip Change	After step complete
Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
Tip Touch	Tip Touch	No

46 Delay

Description:
Incubate RNase-free water on the membrane for 3 min at room temperature

Pipetting settings

Tab	Parameter	Set value
Delay	Delay Time Skip Delay by RUN	180 Yes

Step	Description
47 Message **Description:** Apply vacuum (-0.5 bar, 1 min) If necessary, press down the NucleoSpin RNA Binding Plate slightly until flow through starts	Pipetting settings **Tab**: **Message** **Parameter**: Message Line 1, Message Line 2, Message Line 3 **Set value**: Vacuum, -500 mbar, 1 min
48 Delay **Description:** Allow the buffer to pass the wells	Pipetting settings **Tab**: **Delay** **Parameter**: Delay Time, Skip Delay by RUN **Set value**: 60, Yes
49 Message **Description:** Release vacuum	Pipetting settings **Tab**: **Message** **Parameter**: Message Line 1, Message Line 2, Message Line 3 **Set value**: Release, vacuum, -
50 Message **Description:** Remove the Elution Plate U-bottom containing eluted RNA and seal the plate with adhesive cover foil for further storage	Pipetting settings **Tab**: **Message** **Parameter**: Message Line 1, Message Line 2, Message Line 3 **Set value**: Remove, and store, eluates

Run Protocol

Program Name Program Name (on pipette)	MN RNA TISSUE.iaa MN RNA Tissue
Instrument - Serial Number ASSIST PLUS	
Pipette - Serial Number VIAFLO - 1,250µl - 12CH	
Pipette Tips - Lot Number 1250 µl GripTip, Sterile, Filter	
Notes:	
Run Operator:	
Run Date:	
Run Start Time:	
Run End Time:	
Signature:	