MN RNA CELL Report

Program Name (on pipette)
MN RNA Cells

INTEGRA VIALAB

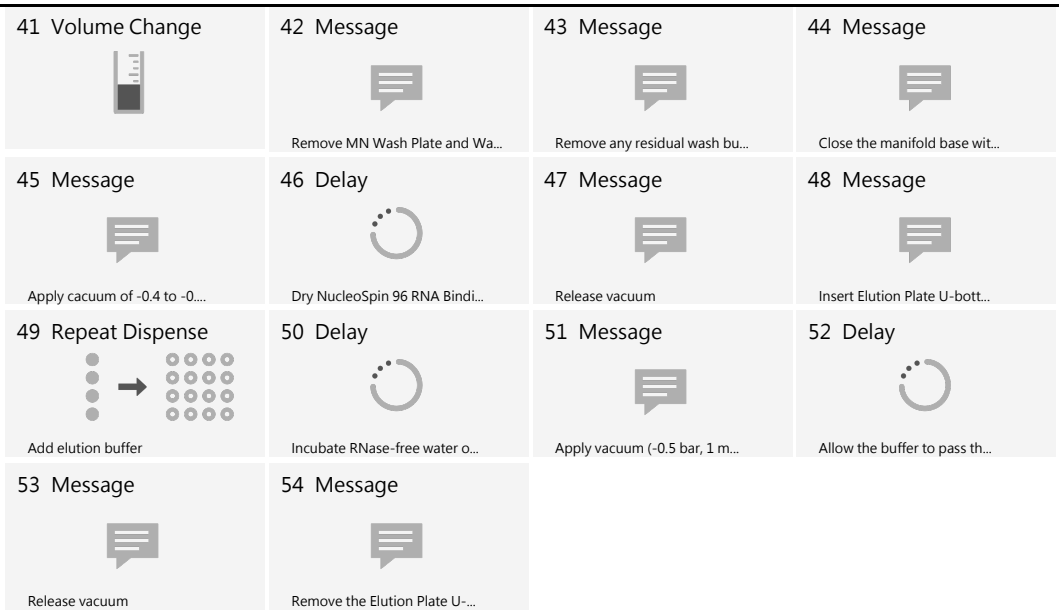
User Credentials

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

Overview Method

VIAFLO - 1,250µl - 12CH

1 Initial Volumes Fill Reservoir 1...	2 Repeat Dispense Lyse cells (Buffer RA1)	3 Message Turn on shaker (1000 rpm)	4 Delay 4 min...
5 Message Turn off shaker	6 Repeat Dispense Add Buffer RA4	7 Transfer Mix and transfer crude lysa...	8 Message Apply vacuum (-0.2 bar, 1 m...
9 Delay Wait until the crude lysate...	10 Message Release vacuum	11 Volume Change	12 Repeat Dispense Add Buffer RA3 to rows 1-4
13 Repeat Dispense Add Buffer RA3 to rows 5-8	14 Message Apply vacuum (-0.2 bar, 1 m...	15 Delay Allow the buffer to pass th...	16 Message Release vacuum
17 Volume Change	18 Repeat Dispense Add rDNase	19 Delay Digest DNA for 15 min	20 Volume Change
21 Repeat Dispense Add Buffer RA2 to rows 1-4,...	22 Repeat Dispense Add Buffer RA2 to rows 5-8,...	23 Message Apply vacuum (-0.2 bar; 1 m...	24 Delay
25 Message Exchange Buffer Rack...	26 Message Release vacuum	27 Volume Change	28 Volume Change
29 Repeat Dispense Add Buffer RA3 to rows 1-3,...	30 Repeat Dispense Add Buffer RA3 to rows 4-6,...	31 Repeat Dispense Add Buffer RA3 to rows 7-8,...	32 Message Apply vacuum (-0.2 bar, 1 m...
33 Delay Allow the buffer to pass th...	34 Message Release vacuum	35 Volume Change	36 Repeat Dispense Add Buffer RA4 to rows 1-4,...
37 Repeat Dispense Add Buffer RA4 to rows 5-8,...	38 Message Apply vacuum (-0,2 bar, 1 m...	39 Delay Allow the buffer to pass th...	40 Message Release vacuum



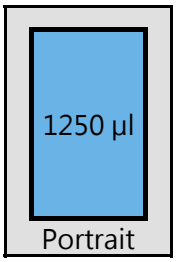
Total Time: 59 min 55 sec

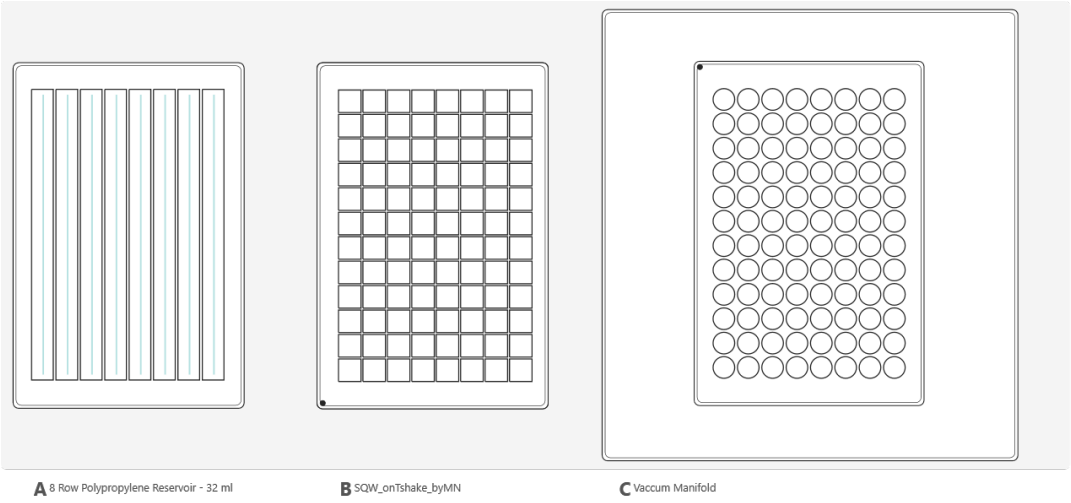
Total Tip Consumption: 192

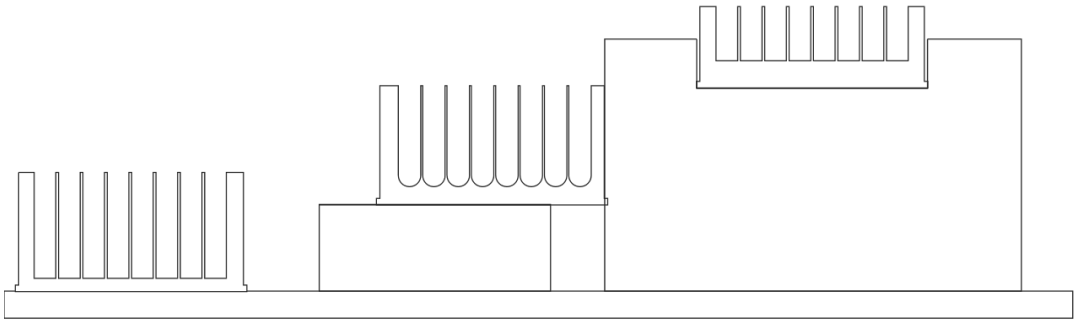
Description

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

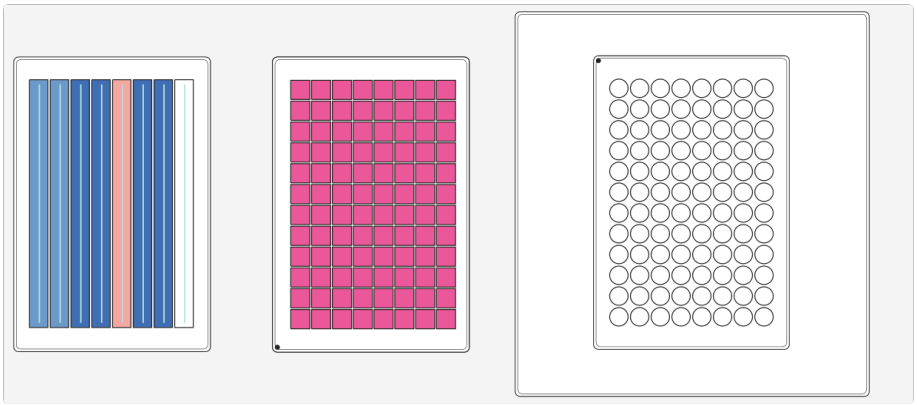
Deck Position	Labware	Name	Manufacturer	Part Number	Description
A	Reservoir	8 Row Polypropylene Reservoir - 32 ml	INTEGRA	6371, 6372	
B	COMBI System	SQW_onTshake_byMN	Macherey Nagel	740481	- shaking device - including F-bottom adapter
	B1	COMBISystem_96 Deepwell U-bottom_Culture Plate_Macherey Nagel	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

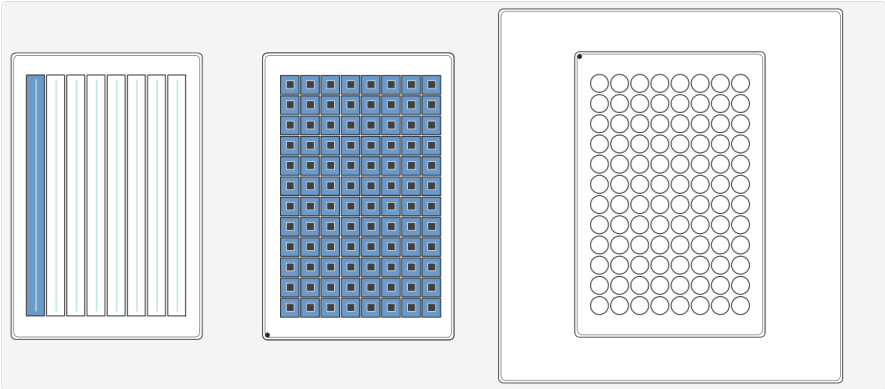
Row A: 15 ml buffer RA1
Row B: 15 ml buffer RA4
Row C and D: 25 ml buffer RA3
Row E: 10 ml rDNase
Row F and G: 25 ml buffer RA2



2 Repeat Dispen...

Time:
1 min 2 sec
Used Tips:
12

Description:
Lyse cells (Buffer RA1)



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	17 mm	B	B1 A12-A1	53.9 mm	130
2	A	A1	17 mm	B	B1 B12-B1	53.9 mm	130
3	A	A1	17 mm	B	B1 C12-C1	53.9 mm	130
4	A	A1	17 mm	B	B1 D12-D1	53.9 mm	130
5	A	A1	17 mm	B	B1 E12-E1	53.9 mm	130
6	A	A1	17 mm	B	B1 F12-F1	53.9 mm	130
7	A	A1	17 mm	B	B1 G12-G1	53.9 mm	130
8	A	A1	17 mm	B	B1 H12-H1	53.9 mm	130

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 40 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 2 mm **Target:** B1: Fix No B1: 0 mm
	Tip Change	Tip Change	After step complete
	Mix	**Source:** Mixing **Target:** Mixing	**Source:** No **Target:** No
	Tip Touch	Tip Touch	No
3 Message **Description:** Turn on shaker (1000 rpm)	Pipetting settings		
	Tab	**Parameter**	**Set value**
	Message	Message Line 1 Message Line 2 Message Line 3	Turn on shaker 1000 rpm
4 Delay **Description:** 4 min Make sure sample is fully resuspended	Pipetting settings		
	Tab	**Parameter**	**Set value**
	Delay	Delay Time Skip Delay by RUN	240 Yes

Step	Description
5 Message	

Description:
Turn off shaker

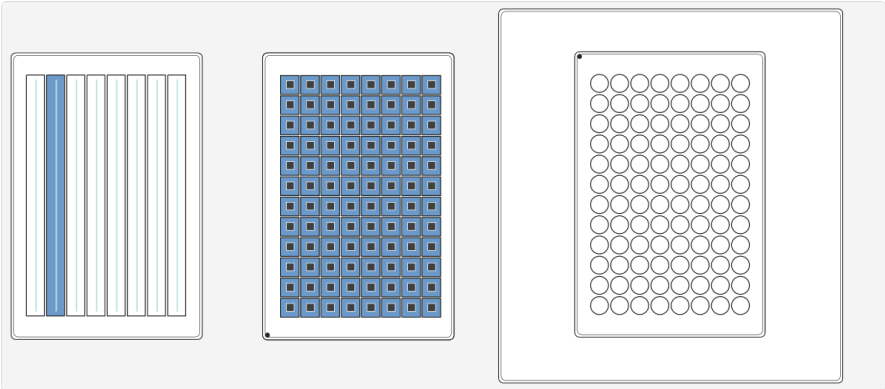
Pipetting settings

Tab	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Turn off shaker -

6 Repeat Dispen...

Time:
1 min 3 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	18.5 mm	B	B1 A12-A1	54.2 mm	130
2	A	A2	18.5 mm	B	B1 B12-B1	54.2 mm	130
3	A	A2	18.5 mm	B	B1 C12-C1	54.2 mm	130
4	A	A2	18.5 mm	B	B1 D12-D1	54.2 mm	130
5	A	A2	18.5 mm	B	B1 E12-E1	54.2 mm	130
6	A	A2	18.5 mm	B	B1 F12-F1	54.2 mm	130
7	A	A2	18.5 mm	B	B1 G12-G1	54.2 mm	130
8	A	A2	18.5 mm	B	B1 H12-H1	54.2 mm	130

Pipetting settings

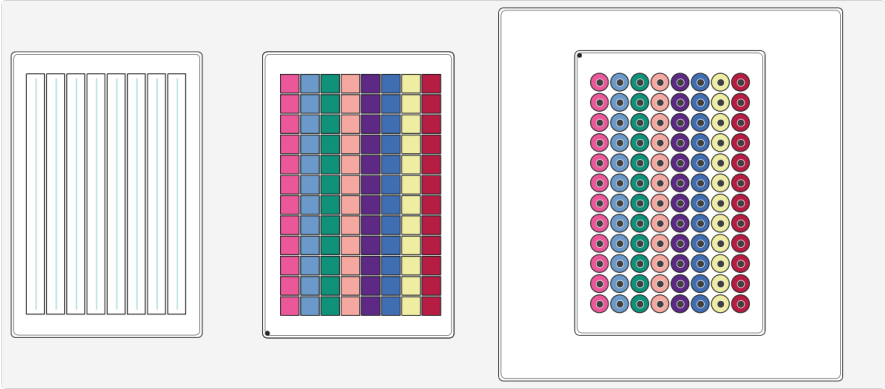
Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** B1: 9 mm
Volumes	Volume Post-Dispense Post-Dispense Location Reuse Post-Dispense Dispense Type Air Gap	Fix 30 µl Source No Multi 30 µl

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

7 Transfer

Time:
5 min 29 sec
Used Tips:
96

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

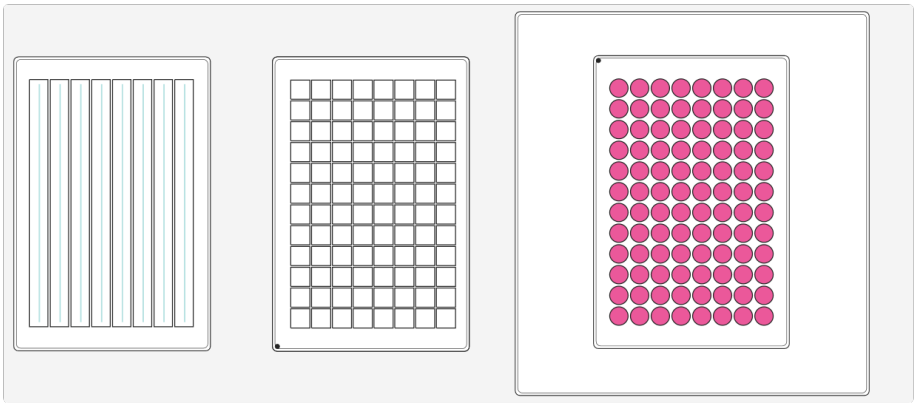
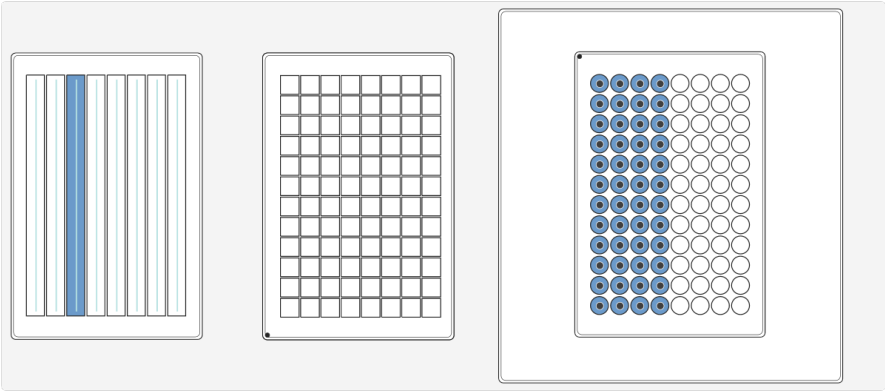


Summary individual transfers

	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	260
2	B	B1 B12-B1	43 mm	C	C1 A2-L2	105 mm	260
3	B	B1 C12-C1	43 mm	C	C1 A3-L3	105 mm	260
4	B	B1 D12-D1	43 mm	C	C1 A4-L4	105 mm	260
5	B	B1 E12-E1	43 mm	C	C1 A5-L5	105 mm	260
6	B	B1 F12-F1	43 mm	C	C1 A6-L6	105 mm	260
7	B	B1 G12-G1	43 mm	C	C1 A7-L7	105 mm	260
8	B	B1 H12-H1	43 mm	C	C1 A8-L8	105 mm	260

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: 200 µl 12 6 No B1: 40 mm **Target:** No
	Tip Touch	Tip Touch	No
8 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
9 Delay **Description:** Wait until the crude lysate has passed the NucleoSpin RNA Bindng Plate	Pipetting settings **Tab**: **Delay** **Parameter**: Delay Time **Set value**: 60 **Parameter**: Skip Delay by RUN **Set value**: Yes
10 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**: -
11 Volume Change	 0 µl
12 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

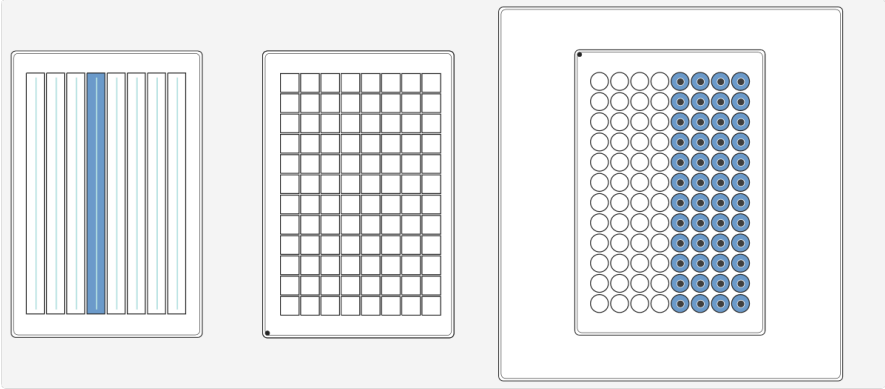
Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Multi 50 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	6 0 2 1 No
Pipetting Height	**Source:** 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

13 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

14 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	Vacuum -200 mbar 3 min

15 Delay

Description:
Allow the buffer to pass the wells

Pipetting settings

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

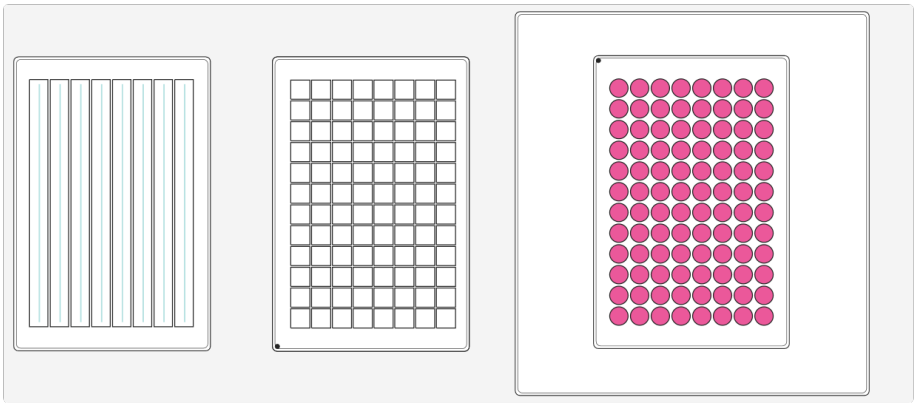
16 Message

Description:
Release vacuum

Pipetting settings

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

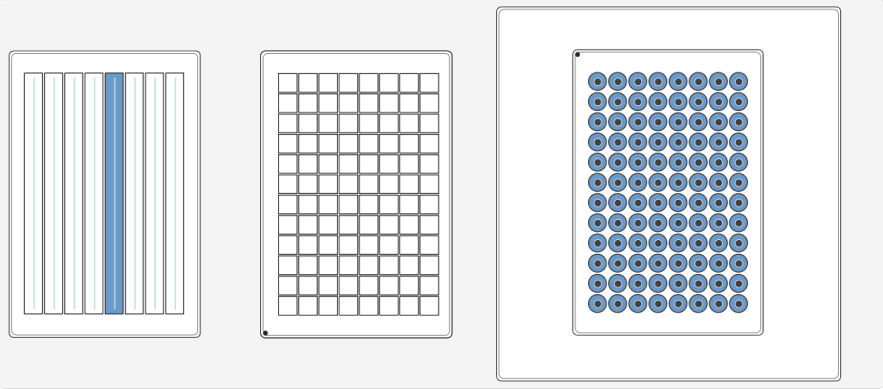
17 Volume Change



Step	Description
18 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

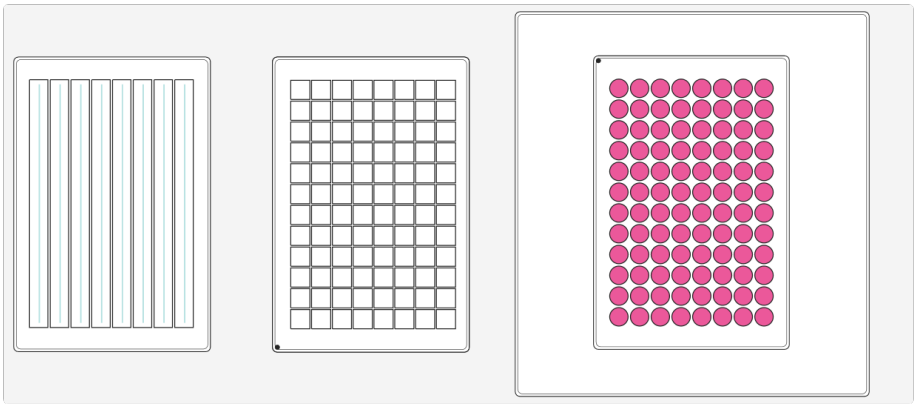
19 Delay

Description:
Digest DNA for 15 min

Pipetting settings

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

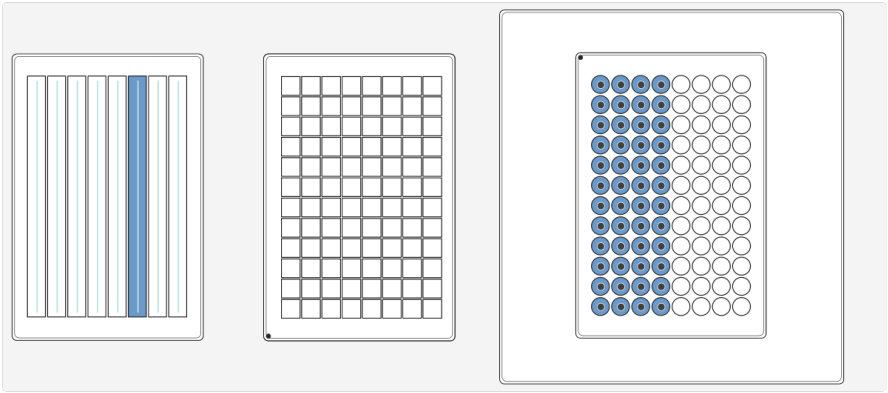
20 Volume Change



21 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

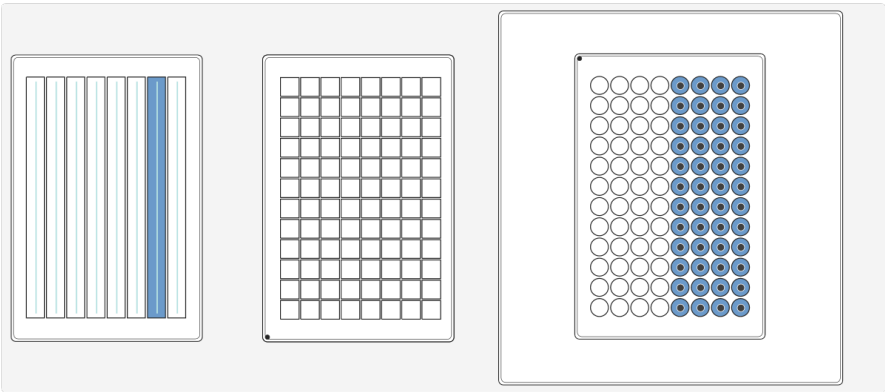
Pipetting settings

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

22 Repeat Dispen...

Time:
0 min 46 sec
Used Tips:
0

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



Summary individual transfers

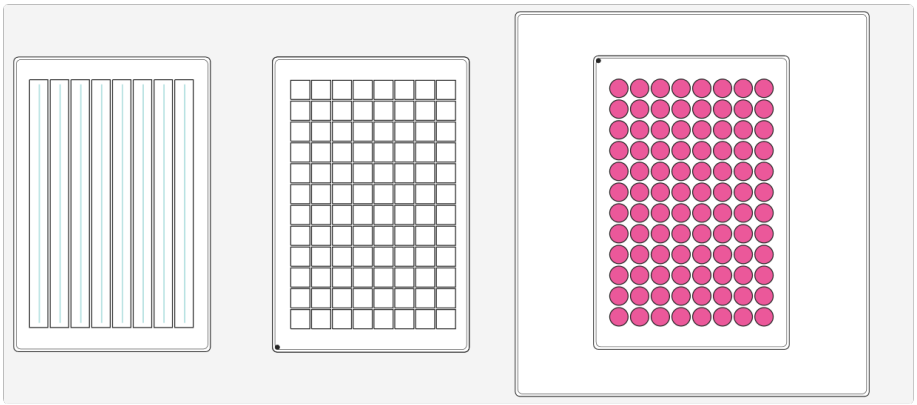
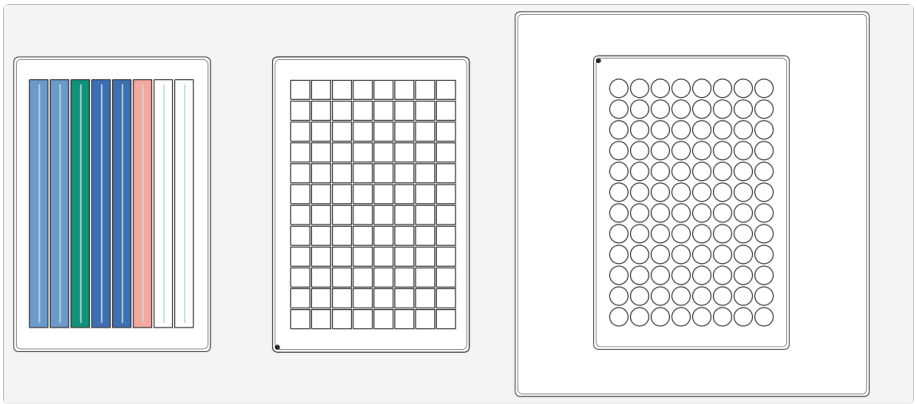
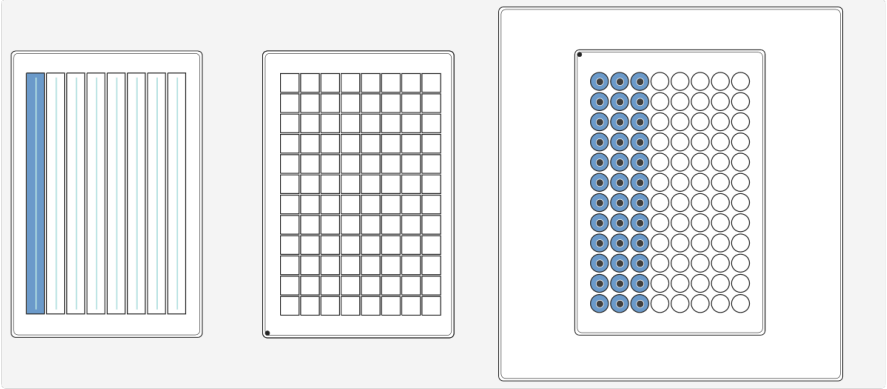
Step	Description

	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
23 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: Vacuum / -200 mbar / 1 min
24 Delay	Pipetting settings Tab: **Delay** Parameter: Delay Time / Skip Delay by RUN Set value: 60 / Yes
25 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 / -
26 Message **Description:** Release vacuum	Pipetting settings Tab: **Message** Parameter: Message Line 1 / Message Line 2 / Message Line 3 Set value: Release / Vacuum / -

Step	Description
27 Volume Change	
28 Volume Change	
29 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

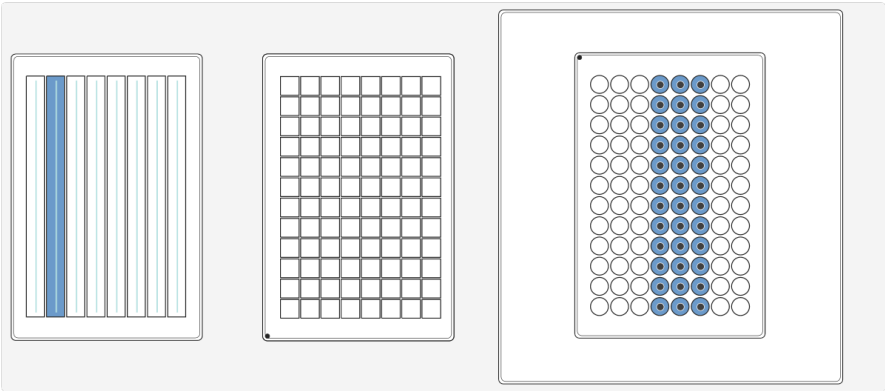
Step	Description

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

30 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

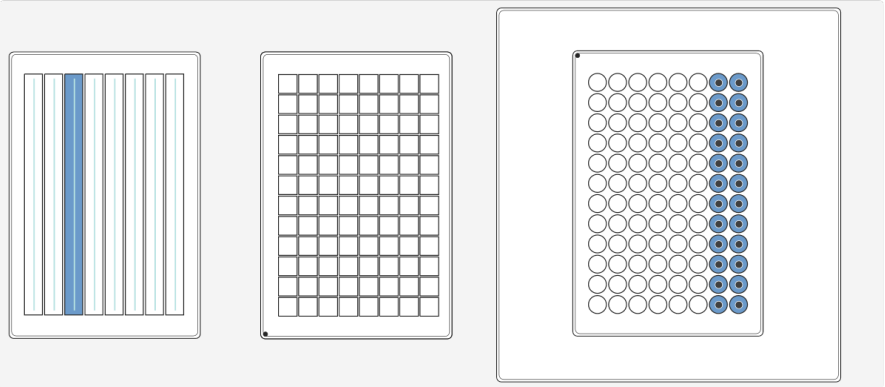
Pipetting settings

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

31 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

	Source			Target			
Step	Deck Position	Well Positions	Start Height [mm]	Deck Position	Well Positions	Start Height [mm]	Volume [µl]
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

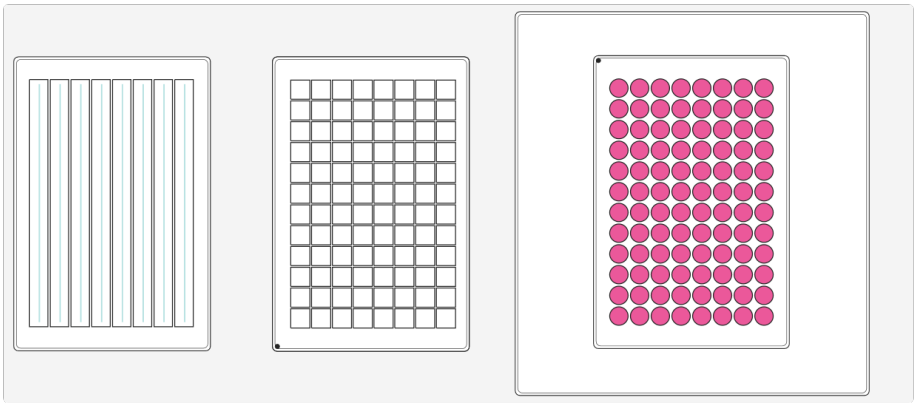
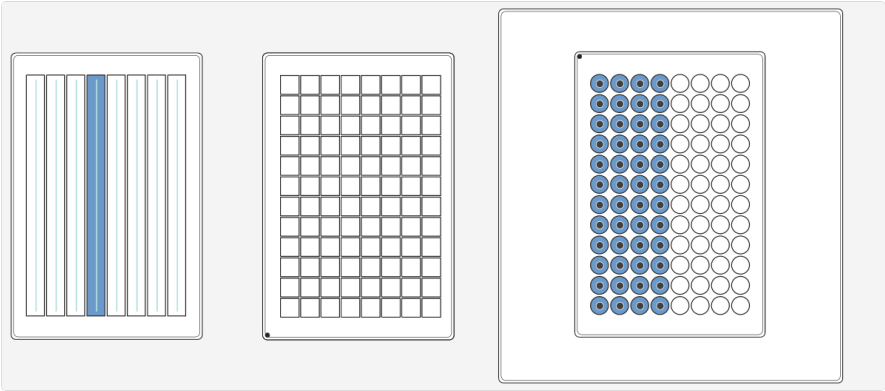
Tab	Parameter	Set value
Pipetting location	**Source:** Tip Spacing **Target:** Tip Spacing	**Source:** 9 mm **Target:** C1: 9 mm
Volumes	Volume Dispense Type Air Gap	Fix Single 40 µl
Pipetting Speeds	Aspiration Speed Aspiration Delay Dispense Speed Dispense Delay Aspirate	5 0 4 0 No
Pipetting Height	**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

32 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
33 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
34 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: -
35 Volume Change	
36 Repeat Dispen... **Time:** 0 min 45 sec **Used Tips:** 12 **Description:** Add Buffer RA4 to rows 1-4, third 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	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

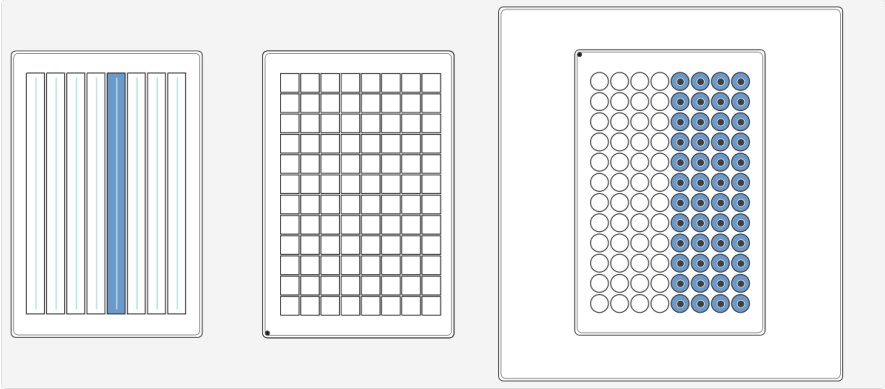
Pipetting settings

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

37 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

38 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

39 Delay

Description:
Allow the buffer to pass the wells

Pipetting settings

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

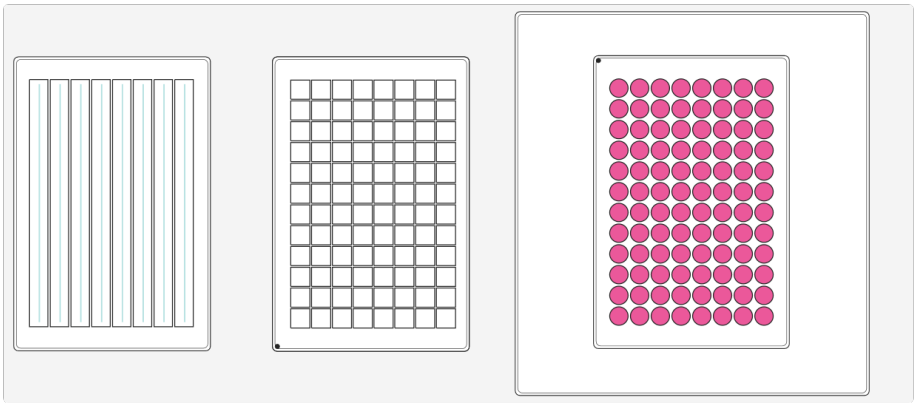
40 Message

Description:
Release vacuum

Pipetting settings

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

41 Volume Change



Step	Description
42 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
43 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
44 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
45 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	Parameter	Set value
Message	Message Line 1 Message Line 2 Message Line 3	Vacuum -600 mbar 12 min

Step	Description
46 Delay **Description:** Dry NucleoSpin 96 RNA Binding Plate	Pipetting settings

Tab	Parameter	Set value
Delay	Delay Time Skip Delay by RUN	720 Yes

Step	Description
47 Message **Description:** Release vacuum	Pipetting settings

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

Step	Description

48 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

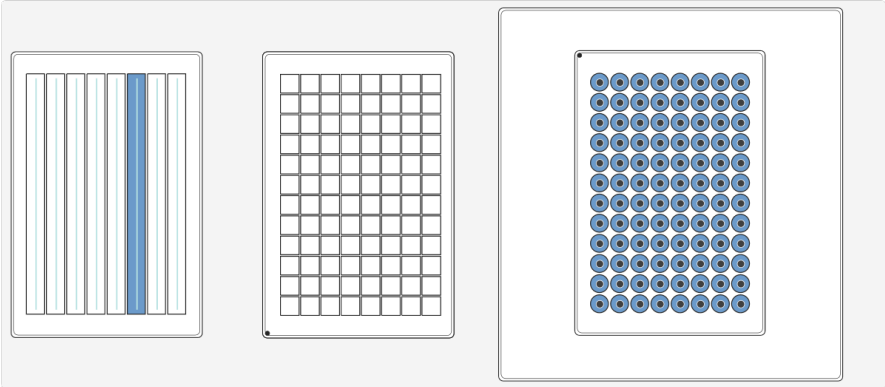
Pipetting settings

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

49 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

50 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
51 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
52 Delay **Description:** Allow the buffer to pass the wells	Pipetting settings Tab: **Delay** Parameter: Delay Time, Skip Delay by RUN Set value: 60, Yes
53 Message **Description:** Release vacuum	Pipetting settings Tab: **Message** Parameter: Message Line 1, Message Line 2, Message Line 3 Set value: Release, vacuum, -
54 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 CELL.iaa MN RNA Cells
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:	