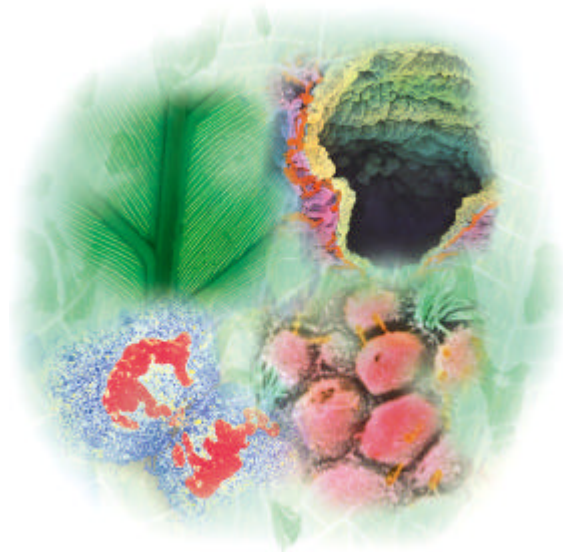


## QuickPick™ SML mRNA

- 41002 • mRNA purification kit, 8 preps
- 41012 • mRNA purification kit, 24 preps
- 41022 • mRNA purification kit, 96 preps



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**1 INTRODUCTION**

These are the instructions for use for the QuickPick™ SML mRNA purification kits. Please read the entire instructions carefully before starting the work. Also refer to the MagRo™ 8-M robotic workstation operating manual or PickPen® 1-M or PickPen® 8-M instructions for use.

QuickPick™ SML mRNA purification kits provide a fast and simple means of purifying poly-A(+) mRNA from cultured cells, total RNA and animal or plant tissues. The method does not require any organic solvents and eliminates the need for repeated centrifugations, vacuum filtration or column separation. The purified mRNA can be used for down-stream applications such as RT-PCR, real-time PCR, cDNA libraries and microarray analyses.

The reagent volumes can be scaled up or down to be used with different sample amounts either with the PickPen® manual tools or with the MagRo™ 8-M robotic workstation.

**1.1 Principle of the method**

QuickPick™ SML mRNA kits are based on the binding of poly-A(+) mRNA to Oligo (dT)<sub>30</sub> coated paramagnetic particles. Sample cells are lysed in the presence of Lysis/Binding Buffer. Cell lysate is incubated with Oligo (dT)<sub>30</sub> coated Magnetic Particles for hybridizing Oligo (dT)<sub>30</sub> to mRNA. Particle-bound mRNA is separated from other RNA species, as well as from other material, by washing. Magnetic Particles are suspended into Elution Buffer and poly-A(+) mRNA is ready for the downstream applications.

**2 SPECIFICATIONS**

Table 1: Specifications for QuickPick™ SML mRNA purification kits.

Sample	Amount	Amount	Amount
Cultured cells	= 10 <sup>3</sup> cells	10 <sup>3</sup> - 10 <sup>5</sup> cells	10 <sup>5</sup> - 10 <sup>6</sup> cells
Animal tissue	= 30 µg	30 µg - 3 mg	3 - 30 mg
Plant tissue	= 50 µg	50 µg - 5 mg	5 - 50 mg
Total RNA	= 50 ng	50 ng - 5 µg	5 - 50 µg
Typical purity <sup>(1)</sup>	Applicable for RT-PCR		

<sup>1</sup>To guarantee that no DNA is present a DNase treatment should be performed prior the downstream applications. For RT-PCR the primers should be designed to anneal at intron splice junctions, which prevents the amplification of DNA.

**3 KIT CONTENTS**

**3.1 Reagents for the SML kits**

Reagent:	41002 <sup>(1)</sup>	41012	41022
Oligo (dT) <sub>30</sub> coated Magnetic Particles	240 µl	0.80 ml	2 x 1.5 ml
Lysis/Binding Buffer <sup>(2,3,4)</sup>	1.6 ml	6 ml	22 ml
Wash Buffer A <sup>(2,3)</sup>	3.2 ml	12 ml	44 ml
Wash Buffer B <sup>(2)</sup>	1.6 ml	6 ml	22 ml
Elution Buffer	1.6 ml	6 ml	22 ml

<sup>1</sup>For manual use only.

<sup>2</sup>Reagent contains LiCl.

<sup>3</sup>Reagent contains LiDS.

<sup>4</sup>Reagent contains DTT.

The reagents for QuickPick™ SML mRNA purification kits can also be bought separately:

Reagent:	Volume:	Product No:
QuickPick™ XL mRNA Oligo (dT) <sub>30</sub> coated Magnetic Particles	12.6 ml	41100
QuickPick™ XL mRNA Lysis/Binding Buffer <sup>(1,2,3)</sup>	83 ml	41300
QuickPick™ XL mRNA Wash Buffer A <sup>(1,2)</sup>	165 ml	41510
QuickPick™ XL mRNA Wash Buffer B <sup>(1)</sup>	83 ml	41520
QuickPick™ XL mRNA Elution Buffer	83 ml	41600

<sup>1</sup>Reagent contains LiCl.

<sup>2</sup>Reagent contains LiDS.

<sup>3</sup>Reagent contains DTT.

### 3.2 Scaling of sample amounts

For both manual and automated protocols the sample amounts can be scaled yielding to different number of preparations (Table 2) and reagent consumption. The reagent volumes are linearly dependent on the used sample amount. The reagent volumes for the manual purifications are shown in Tables 3 and 4 (see Chapter 6 "Protocols for manual PickPen® 1-M and 8-M tools"). In MagRo™ 8-M robotic workstation protocols the reagent volumes are adjusted to be suitable for automation.

Table 2: The effect of sample amount to number of preparations for the purifications with QuickPick™ mRNA SML kits.

Sample	Amount of sample per preparation		
Cultured cells	= 10 <sup>3</sup> cells	10 <sup>3</sup> - 10 <sup>5</sup> cells	10 <sup>5</sup> - 10 <sup>6</sup> cells
Animal Tissue	= 30 µg	30 µg - 3 mg	3 - 30 mg
Plant Tissue	= 50 µg	50 µg - 5 mg	5 - 50 mg
Total RNA	= 50 ng	50 ng - 5 µg	5 - 50 µg
<b>Sample Volume in Lysis/Binding Buffer</b>	100 µl	200 µl	400 µl
<b>Number of preps:</b>			
41002 <sup>(1)</sup>	16	8	4
41012	48	24	12
41022	192	96	48

<sup>1</sup>For manual use only.

## 4 SAMPLE PREPARATION

The user should utilize a sample preparation method that is known to yield undegraded RNA. The use of RNA stabilization solutions is recommended in order to ensure intact mRNA during sample preparation.

Direct mRNA purification from cells or tissues may yield small amounts of rRNA which does not affect the function of mRNA.

### 4.1 Sample preparation from cultured cells

Amount of mRNA in cultured cells is dependent on the cell type, development stage and growth conditions.

Harvest the needed amount of cultured cells by centrifuging for 5 minutes at 300 x g using RNase-free centrifuge tube. Remove the supernatant carefully. Wash the cells once with PBS and centrifuge again for 5 minutes at 300 x g. Suspend cell pellet by adding appropriate volume of Lysis/Binding Buffer (see Table 2). Mix the sample by vortexing or pipetting up and down several times. The mixing of cells with Lysis/Binding Buffer forms foam, which can be eliminated by a brief centrifugation (e.g. 2 minutes at 10,000 x g).

The viscosity of the sample can be reduced according to Chapter 4.4 "Reducing of sample viscosity by homogenization".

### 4.2 Sample preparation from animal or plant tissue

Complete disruption of cell walls, plasma membranes, and organelle membranes is essential to release all the nucleic acids from the tissue. Insufficient disruption of starting material will lead to low mRNA yield. Cell wall properties vary widely between species and proper homogenization method should be applied to achieve complete disruption. The disruption can be performed for example by mechanical grinding (Pellet Pestle or equivalent device) or with liquid nitrogen using mortar and pestle. Other disruption methods can also be used.

With plants it is preferable to harvest young plant material (e.g. expanding leaves or needles). mRNA yields from young plant tissues are often higher than from old plant tissue, because young plant tissue generally contains more cells than the same amount of older plant tissue. In addition, young plant tissue contains fewer metabolites (such as polyphenolics, polysaccharides and flavones) which may affect the performance of the downstream applications.

#### Disruption by mechanical grinding

Mechanical grinding disrupts samples efficiently and helps in rapid preparation of the sample homogenate. Weight the sample into 2 ml RNase-free tube. Add appropriate volume of Lysis/Binding Buffer (Table 2) and disrupt the sample manually or with Pellet Pestle or equivalent device. A homogeneous suspension should be obtained within 5 - 10 minutes. Keep the disrupted sample on ice. The Lysis/Binding Buffer forms foam which can be eliminated by a brief centrifugation (e.g. 2 minutes at 10,000 x g). Use the supernatant as the sample.

The viscosity of the sample can be reduced according to Chapter 4.4 "Reducing of sample viscosity by homogenization".

#### Disruption with liquid nitrogen using mortar and pestle

One of the most common disruption methods involves freezing samples in liquid nitrogen and grinding with a mortar and pestle.

1. Freeze the sample in liquid nitrogen immediately after harvesting. Do not let the sample to thaw at any time during disruption.
2. Pre-cool equipments by pouring liquid nitrogen into mortar and placing the pestles grinding end in the liquid nitrogen.
3. Place frozen sample in mortar and grind until fine whitish powder results.
4. Add liquid nitrogen as necessary but be careful not to spill the sample out of the mortar.
5. Using a pre-cooled spatula transfer the powdered sample into pre-cooled tubes. Use several tubes for large samples to avoid thawing.
6. Ensure all the liquid nitrogen has evaporated before closing the tube.
7. If the sample is not processed immediately the tube should be kept on dry ice or liquid nitrogen or stored at -80°C, to prevent the sample from thawing.
8. If the sample is processed immediately after homogenization, add correct volume of Lysis/Binding Buffer (Table 2) before the sample thaws.
9. Mix the sample by vortexing or pipetting up and down several times. Lysis/Binding Buffer forms foam which can be eliminated by a brief centrifugation (e.g. 2 minutes at 10,000 x g). Use the supernatant as the sample.

The viscosity of the sample can be reduced according to Chapter 4.4 "Reducing of sample viscosity by homogenization".

### 4.3 Sample preparation from total RNA

Use appropriate amount of total RNA (Table 2). Adjust the final volume with Lysis/Binding Buffer. Mix the sample by vortexing or pipetting up and down several times. Lysis/Binding Buffer forms foam which can be eliminated by brief centrifugation (e.g. 2 minutes at 10,000 x g).

### 4.4 Reducing of sample viscosity by homogenization

The viscosity of cell lysate can be reduced by homogenization using needle and syringe, as follows: Pass the cell lysate through a 19-G needle attached to a 1 ml or 2 ml syringe until a homogeneous lysate is achieved. The homogenization of the cell lysate through a needle results in foaming which can be eliminated by brief centrifugation (e.g. 2 minutes at 10,000 x g). The centrifugation step may yield a pellet containing cell debris. Use the supernatant as the sample.

## 5 PICKPEN® TIPS

The PickPen® tips packed in bulk quantities in plastic bags are not RNase-free. To eliminate RNase activity the tips should be baked (see Chapter 5.1 below) provided that they are removed from the plastic bag. The separately available PickPen® Tip box can be autoclaved but not baked.

### 5.1 Eliminating RNases from PickPen® tips

Place the PickPen® tips in glass beaker so that the heads of the tips are towards the bottom of the beaker. Cover the beaker with aluminum foil. Bake tips at 160°C – 180°C for at least 4 h or overnight. Transfer the tips into autoclaved PickPen® tip box using RNase-free forceps in RNase-free environment. Close the PickPen® tip box and store at room temperature.

## 6 PROTOCOLS FOR MANUAL PICKPEN® 1-M AND 8-M TOOLS

### 6.1 PickPen® 1-M protocol

#### Notes

1. All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved.
2. Oligo (dT)<sub>30</sub> coated Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended.
3. Repeat pipettors should not be used when dispensing Magnetic Particles.
4. RNase-free water can also be used for elution.
5. Maintain RNase-free conditions while working with RNA.
6. β-mercaptoethanol can be used in Lysis/Binding Buffer for inhibition of RNases. Add 10 μl β-mercaptoethanol in 1 ml Lysis/Binding Buffer.
7. The purified mRNA may contain chromosomal DNA. If elimination of chromosomal DNA is required for the downstream application DNase I treatment should be carried out.
8. If the purified mRNA is to be used in an enzymatic downstream application (for example RT-PCR), an additional quick washing step with the 1x downstream buffer (not included in the kit) may be carried out after Wash Buffer B. This ensures the removal of residual LiDS which may inhibit the reverse transcriptase activity.
9. The mRNA can be eluted from Magnetic Particles by using a heating block (+70°C) or an equivalent device. Proceed as follows: Collect the Magnetic Particles from the Wash Buffer B and release them into Elution Buffer and mix thoroughly. Elute the mRNA from the Magnetic Particles by heating at +70°C for 5 minutes. While poly-A(+) mRNA is eluted from the Magnetic Particles

the PickPen® Tip may be detached from the PickPen® and stored in Wash Buffer B. After elution collect the Magnetic Particles from Elution Buffer and discard them and the tip. The eluate can be used directly for downstream applications or stored at -80°C.

10. Plant tissues often contain polysaccharides and polyphenols. These compounds may interfere with for example RT-PCR reactions. When using solid-phase (particle-bound) mRNA from plant tissue as a template in RT-PCR, we recommend using smaller amounts of the template than usual.

### Reagent volumes

Table 3: Reagent volumes for PickPen® 1-M purifications.

Reagent	Reagent volume per preparation		
	= 10 <sup>3</sup> cells	10 <sup>3</sup> - 10 <sup>5</sup> cells	10 <sup>5</sup> - 10 <sup>6</sup> cells
Lysis/Binding Buffer	100 μl	200 μl	400 μl
Magnetic Particles	15 μl	30 μl	60 μl
Wash Buffer A	2 x 100 μl	2 x 200 μl	2 x 400 μl
Wash Buffer B	100 μl	200 μl	300 μl
Elution Buffer	5 - 50 μl	10 - 50 μl	25 - 50 μl

<sup>1</sup>Cultured cells used as an example, for the amounts of other sample materials, see Table 2.

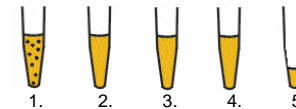
### Materials required

1. RNase-free 1.5 – 2.0 ml tubes.
2. Pipettes and RNase-free aerosol resistant micropipettor tips.
3. PickPen® 1-M tool and RNase-free PickPen® tips.
4. Optional: Syringe and needle (for homogenization viscous cell lysates).
5. Optional: Heating block (+70°C) for elution of mRNA into solution (see Note 9 above).

### Protocol

1. Prepare the sample according to Chapter 4 "Sample Preparation".
2. Number tubes from 1 to 5. Pipette appropriate volumes of QuickPick™ SML mRNA purification reagents (according to the Table 3) into tubes 1 - 5 as follows:

Tube 1: Sample in Lysis/Binding Buffer and Oligo (dT)<sub>30</sub> coated Magnetic Particles  
Tube 2: Wash Buffer A  
Tube 3: Wash Buffer A  
Tube 4: Wash Buffer B  
Tube 5: Elution Buffer



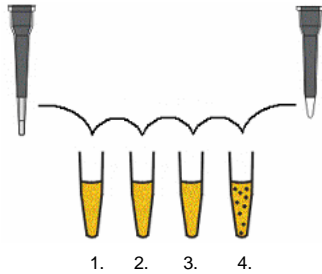
All reagents and the sample should be kept at room temperature during the mRNA purification procedure.

- Mix the tube 1 thoroughly but gently (e.g. with a tube rotator) at room temperature for 5 minutes, and allow mRNA to anneal to the Magnetic Particles. Do not vortex.



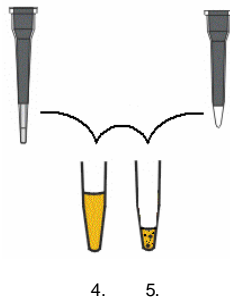
Incubate for 5 minutes with gentle mixing at room temperature

- Pick up the PickPen<sup>®</sup> tip with the PickPen<sup>®</sup> 1-M. Extend the magnet 2 - 3 times to check that the tip is firmly in place. Collect the Magnetic Particles from tube 1 with PickPen<sup>®</sup> 1-M and release them into tube 2 (Wash Buffer A). Wash the Magnetic Particles by mixing the suspension gently for 5 - 15 seconds using the PickPen<sup>®</sup> tip. Note that the magnet has to be withdrawn at this point. Repeat the washing steps in tubes 3 and 4 (Wash Buffer A and Wash Buffer B).



Washing steps

- Collect the Magnetic Particles from tube 4 with PickPen<sup>®</sup> 1-M and release them into tube 5 (Elution Buffer) and mix thoroughly. Place the tube on ice. The purified mRNA stay bound on the Magnetic Particles (solid phase elution) and is ready for downstream applications. Use a correct volume (dependent on the sample amount) of the mixed suspension for the downstream application. Alternatively the Magnetic Particle suspension with bound mRNA can be stored at -80°C for later use.



Solid phase elution

## 6.2 PickPen<sup>®</sup> 8-M protocol

### Notes

- All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved.
- Oligo (dT)<sub>30</sub> coated Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended.
- Repeat or 8-channel pipettors should not be used when dispensing Magnetic Particles.
- When using 96-well plates, the use of an orbital shaker is recommended. Adjust the speed to the highest possible level without causing liquid spill but still keeping Magnetic Particles in suspension.
- RNase-free water can also be used for elution.
- Maintain RNase-free conditions while working with RNA.
- β-mercaptoethanol can be used in Lysis/Binding Buffer for inhibition of RNases. Add 10 μl β-mercaptoethanol in 1 ml Lysis/Binding Buffer.
- The purified mRNA may contain chromosomal DNA. If elimination of chromosomal DNA is required for the downstream application DNase I treatment should be carried out.
- If the purified mRNA is to be used in an enzymatic downstream application (for example RT-PCR), an additional quick washing step with the 1x downstream buffer (not included in the kit) may be carried out after Wash Buffer B. This ensures the removal of residual LiDS which may inhibit the reverse transcriptase activity.
- The mRNA from Magnetic Particles can be eluted by using a heating block (+70°C) or an equivalent device. Proceed as follows: Collect the Magnetic Particles from the Wash Buffer B and release them into Elution Buffer and mix thoroughly. Elute the mRNA from the Magnetic Particles by heating at +70°C for 5 minutes. After elution collect the Magnetic Particles from Elution Buffer and discard them and the tips. The eluates can be used directly for downstream applications or stored at -80°C for later use.
- Plant tissues often contain polysaccharides and polyphenols, which may interfere with RT-PCR reactions. When using solid-phase (particle-bound) mRNA from plant tissue as a template in RT-PCR, we recommend using smaller amounts of the template than usual.

### Reagent volumes

Table 4: Reagent volumes for PickPen<sup>®</sup> 8-M purifications.

Reagent	Reagent volume per preparation		
	Sample amount <sup>(1)</sup> = 10 <sup>3</sup> cells	10 <sup>3</sup> - 10 <sup>5</sup> cells	10 <sup>5</sup> - 10 <sup>6</sup> cells
Lysis/Binding Buffer	100 μl	200 μl	400 μl
Magnetic Particles	15 μl	30 μl	60 μl
Wash Buffer A	2 x 100 μl	2 x 200 μl	2 x 400 μl
Wash Buffer B	100 μl	200 μl	300 μl
Elution Buffer	5 - 50 μl	10 - 50 μl	25 - 50 μl

<sup>1</sup>Cultured cells used as an example, for the amounts of other sample materials, see Table 2.

### Materials required

- Sterile U-bottom 96-well plates (see Table 5).
- Pipettes and RNase-free aerosol resistant micropipettor tips.
- PickPen<sup>®</sup> 8-M tool and RNase-free PickPen<sup>®</sup> tips in tip box.
- Orbital shaker for 96-well plates.

- Optional: Syringes and needles (for homogenization viscous cell lysates).
- Optional: Heating block (+70°C) for elution of mRNA into solution. (see Note 10 above).

Table 5: Recommended plates for different sample amounts.

Sample amount <sup>(1)</sup>	Recommended plate	Bio-Nobile Product No.
= 10 <sup>3</sup> cells	Nunc 96-well microplate, 300 µl Sterile	M1-262126, 10 plates M-262162, 50 / case
10 <sup>3</sup> – 10 <sup>5</sup> cells	Nunc 96-well microplate, 500 µl Sterile	M1-267334, 10 plates M-267334, 120 / case
10 <sup>5</sup> – 10 <sup>6</sup> cells	Nunc 96 deep-well, 1 ml Sterile	M1-260251, 10 plates M-260251, 50 / case

<sup>1</sup>Cultured cells used as an example, for the amounts of other sample materials, see Table 2.

## Protocol

The following instructions are for 8 samples. Samples are prepared in tubes and transferred into 96-well plates (U-bottom) where the rest of the protocol is carried out. The optional elution step can also be performed in a heating block or an equivalent device suitable for 96-well plates.

- Prepare the samples (number tubes from 1 to 8) according to Chapter 4 "Sample preparation".
- Pipette QuickPick™ SML mRNA purification reagents (according to the Table 4) into 96-well plate columns 1 - 5 as follows:

- Column 1: Sample in Lysis/Binding Buffer  
and Oligo (dT)<sub>30</sub> coated Magnetic Particles
- Column 2: Wash Buffer A
- Column 3: Wash Buffer A
- Column 4: Wash Buffer B
- Column 5: Elution Buffer



All reagents and the samples should be kept at room temperature during the mRNA purification procedure.

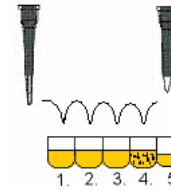
- Mix the 96-well plate on the orbital shaker for 5 minutes at room temperature. Make sure that the Magnetic Particles are in suspension during this step.



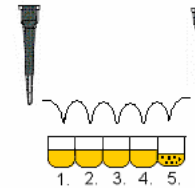
Incubate for 5 minutes at room temperature with continuous mixing

- Pick up the PickPen® tips using PickPen® 8-M. Extend the magnets 2 - 3 times to check that the tips are firmly in place. Collect the Magnetic Particles from column 1 with PickPen® 8-M and release them into column 2 (Wash Buffer A). Mix the suspensions gently for 5 – 15 seconds using

the PickPen® tips. Note that the magnets have to be withdrawn at this point. Repeat the washing steps in columns 3 and 4 (Wash Buffer A and Wash Buffer B).



- Collect the Magnetic Particles from column 4 (Wash Buffer B) with PickPen® 8-M and release them into column 5 (Elution Buffer) and mix thoroughly. Place the plate on ice. The purified mRNA stay bound on the Magnetic Particles (solid phase elution) and is ready for downstream applications. Use an appropriate volume (dependent on the sample amount) of the mixed suspension for the downstream application. Alternatively the Magnetic Particle suspension with bound mRNA can be stored at -80°C for later use.



Solid phase elution

## 7 PROTOCOLS FOR MAGRO™ 8-M ROBOTIC WORKSTATION

### 7.1 Notes for working with MagRo™ 8-M

- All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved.
- Treat all surfaces with RNase decontamination solution to maintain RNase-free conditions.
- Before starting any process make sure that:
  - You have enough disposable RNase-free aerosol resistant tips (DiTi) for pumps.
  - You have enough disposable RNase-free tips for the AutoPickPen™ (APP).
  - You have enough reagents in the reagent bottles.
  - Waste box is empty.
  - The APP and the gripper are correctly positioned in their place.
  - There are no tips attached on APP or the pumps.
- See also MagRo™ 8-M manual for proper use of the instrument. There are more instructions on how to use the software and how to execute processes.
- RNase-free water can also be used for elution.
- β-mercaptoethanol can be used in Lysis/Binding Buffer for inhibition of RNases. Add 10 µl β-mercaptoethanol in 1 ml Lysis/Binding Buffer.
- Use sterile U-bottom 96-well plates for purifications (see Table 5).

## 7.2 Executing MagRo™ 8-M protocols (macros)

1. Switch on the instrument and start the Lirix software.

2. Each macro has an individual name. Look for correct macro name in the Table 6. To find a macro from MagRo™ 8-M open Lirix Shortcuts and click Start Macro icon.



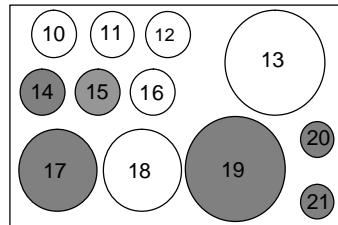
Table 6: The names of the macros for MagRo™ 8-M used with QuickPick™ SML mRNA purification kits.

Preps	Macro names		
	$\leq 10^3$ cells <sup>(1)</sup>	$10^3 - 10^5$ cells <sup>(1)</sup>	$10^5 - 10^6$ cells <sup>(1)</sup>
1 – 24	mRNA_S_24prep (Chapter 7.3)	mRNA_M_24prep (Chapter 7.6)	mRNA_L_24prep (Chapter 7.9)
25 – 48	mRNA_S_48prep (Chapter 7.4)	mRNA_M_48prep (Chapter 7.7)	mRNA_L_48prep (Chapter 7.10)
49 – 96	mRNA_S_96prep (Chapter 7.5)	mRNA_M_96prep (Chapter 7.8)	Not available

<sup>(1)</sup>Cultured cells used as an example, for the amounts of other sample materials, see Table 2.

- Click the correct macro name in “Macro Names” –list to open the process layout in the window.
- Place trays, tip boxes, 96-well plates on a deck and racks as described in Chapters 7.3 to 7.10.
- Mix all reagent bottles, open and place them in the Reagent rack in following positions:

Positions 20 and 21: Oligo (dT)<sub>30</sub> Magnetic Particles  
 Position 19: Wash Buffer A  
 Position 17: Wash Buffer B  
 Position 14 and 15: Elution Buffer



**NOTE:** If preparing up to 48 samples, only one bottle of each reagent is needed for the run (place them in positions 14, 17, 19 and 20).

- Prepare samples according to Chapter 4: “Sample preparation”. Use appropriate volume of Lysis/Binding Buffer (Table 2) for the sample. Mix the samples thoroughly and pipette them into Sample plate, starting from column 1. Place the Sample plate as described in Chapters 7.3 to 7.10.
- Start the macro by double-clicking the name.
- A dialog window opens, in which you may enter the run specific information. You can also choose to start with refilled DiTi boxes by ticking the box. Press Next to continue.
- Macro asks you to verify that all the items placed on the deck correspond to the displayed layout. Press Start Run to continue.
- A dialog window asking for number of samples and other variables appears. Enter the number of samples and press OK. MagRo™ 8-M starts the macro.

11. After finishing the macro a Message box appears notifying that the run is finished. Click OK.

12. The eluates containing the purified mRNA bound to Magnetic Particle (deck tray 4 at position 3) are ready to be used for downstream applications.

13. If the purified mRNA is not used on the same day, store at -20°C until use.

### 7.3 MagRo™ 8-M purification from = 10<sup>3</sup> cultured cells, 1 - 24 preps

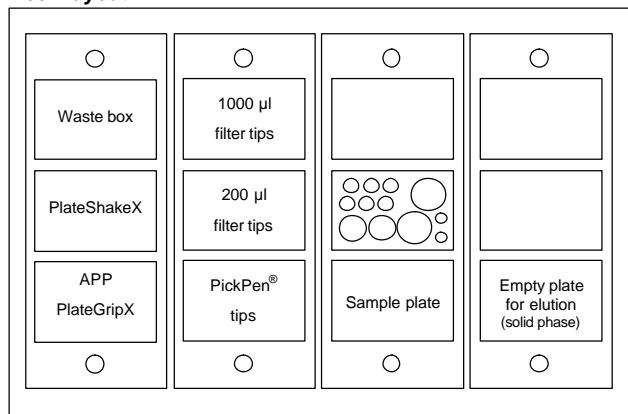
#### Specifications

Sample material amount: = 10<sup>3</sup> cultured cells  
 Preps in one run: 1 – 24  
 Macro name: mRNA\_S\_24

#### Materials required

1. Samples in a 96-well microplate, 300µl
2. One sterile 96-well microplate, 300µl
3. Reagent rack
4. RNase-free aerosol resistant Rainin 200 µl and 1000 µl tips in DiTi boxes
5. RNase-free PickPen® tips in 96 tip box

#### Deck layout



### 7.4 MagRo™ 8-M purification from = 10<sup>3</sup> cultured cells, 25 - 48 preps

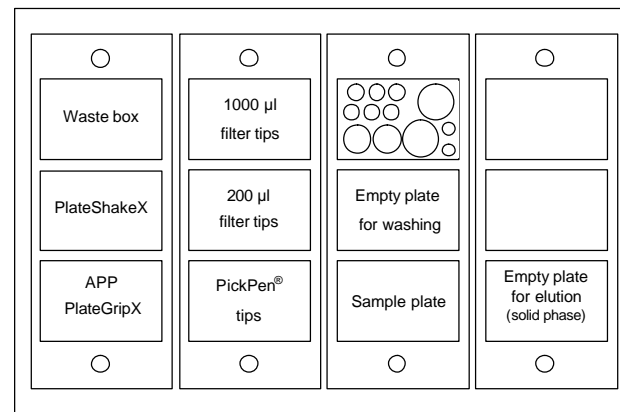
#### Specifications

Sample material amount: = 10<sup>3</sup> cultured cells  
 Preps in one run: 25 – 48  
 Macro name: mRNA\_S\_48

#### Materials required

1. Samples in a 96-well microplate, 300 µl
2. Two sterile 96-well microplates, 300 µl
3. Reagent rack
4. RNase-free aerosol resistant Rainin 200 µl and 1000 µl tips in DiTi boxes
5. RNase-free PickPen® tips in 96 tip box

#### Deck layout



### 7.5 MagRo™ 8-M purification from $10^3$ cultured cells, 49 - 96 preps

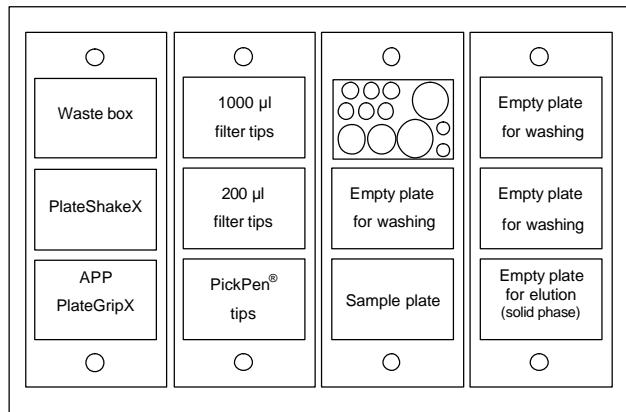
#### Specifications

Sample material amount: =  $10^3$  cultured cells  
 Preps in one run: 49 - 96  
 Macro name: mRNA\_S\_96

#### Materials required

1. Samples in a 96-well microplate, 300  $\mu$ l
2. Four sterile 96-well microplates, 300  $\mu$ l
3. Reagent rack
4. RNase-free aerosol resistant Rainin 200  $\mu$ l and 1000  $\mu$ l tips in DiTi boxes
5. RNase-free PickPen® tips in 96 tip box

#### Deck layout



### 7.6 MagRo™ 8-M purification from $10^3$ - $10^5$ cultured cells, 1 - 24 preps

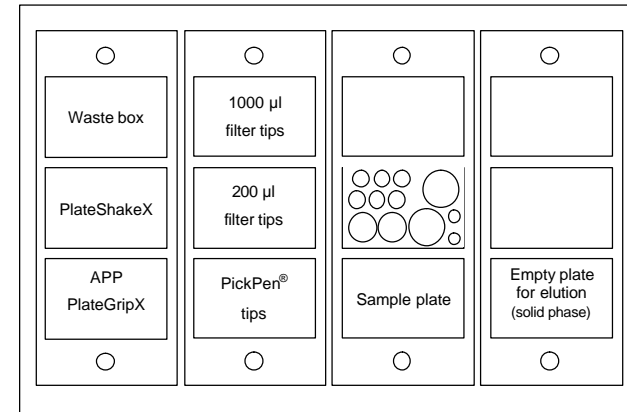
#### Specifications

Sample material amount:  $10^3$  -  $10^5$  cultured cells  
 Preps in one run: 1 - 24  
 Macro name: mRNA\_M\_24

#### Materials required

1. Samples in a 96-well microplate, 500  $\mu$ l
2. One sterile 96-well microplate, 300  $\mu$ l for elution
3. Reagent rack
4. RNase-free aerosol resistant Rainin 200  $\mu$ l and 1000  $\mu$ l tips in DiTi boxes
5. RNase-free PickPen® tips in 96 tip box

#### Deck layout



### 7.7 MagRo™ 8-M purification from 10<sup>3</sup> - 10<sup>5</sup> cultured cells, 25 - 48 preps

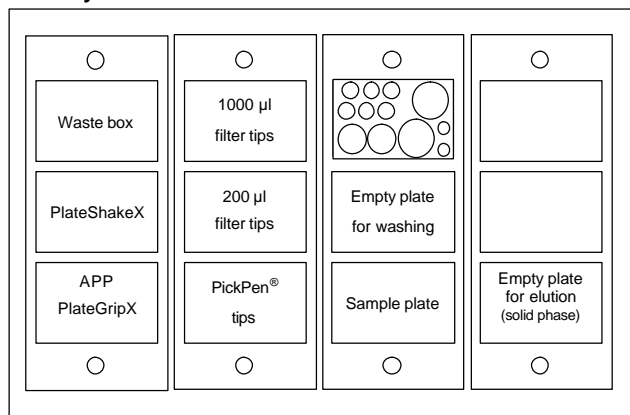
#### Specifications

Sample material amount: 10<sup>3</sup> - 10<sup>5</sup> cultured cells  
 Preps in one run: 25 – 48  
 Macro name: mRNA\_M\_48

#### Materials required

1. Samples in a 96-well microplate, 500 µl
2. One sterile 96-well microplate, 500 µl for washing
3. One sterile 96-well microplate, 300 µl for elution
4. Reagent rack
5. RNase-free aerosol resistant Rainin 200 µl and 1000 µl tips in DITi boxes
6. RNase-free PickPen® tips in 96 tip box

#### Deck layout



### 7.8 MagRo™ 8-M purification from 10<sup>3</sup> - 10<sup>5</sup> cultured cells, 49 – 96 preps

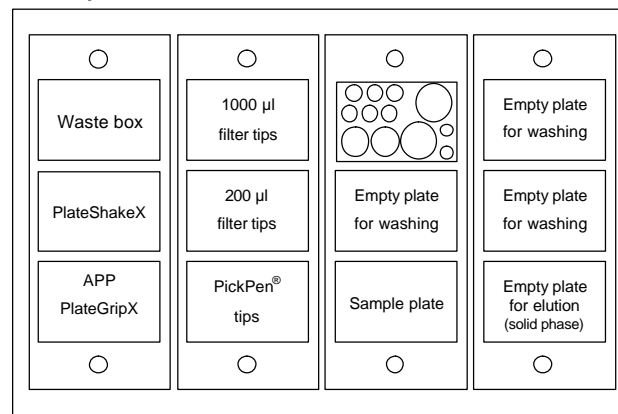
#### Specifications

Sample material amount: 10<sup>3</sup> - 10<sup>5</sup> cultured cells  
 Preps in one run: 49 – 96  
 Macro name: mRNA\_M\_96

#### Materials required

1. Samples in a 96-well microplate, 500 µl
2. Three sterile 96-well microplates, 500 µl for washing
3. One sterile 96-well microplate, 300 µl for elution
4. Reagent rack
5. RNase-free aerosol resistant Rainin 200 µl and 1000 µl tips in DITi boxes
6. RNase-free PickPen® tips in 96 tip box

#### Deck layout



## 7.9 MagRo™ 8-M purification from 10<sup>5</sup> – 10<sup>6</sup> cultured cells, 1 - 24 preps

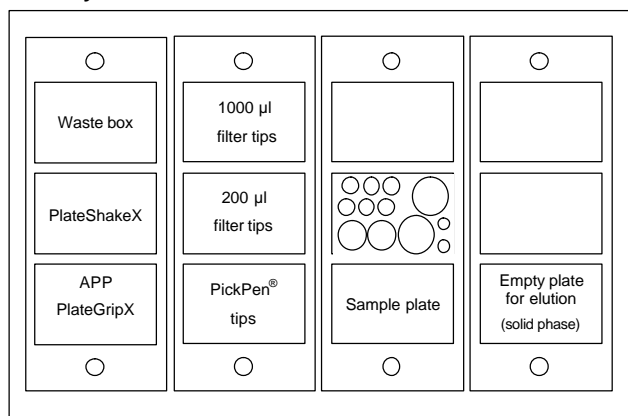
### Specifications

Sample material amount: 10<sup>5</sup> - 10<sup>6</sup> cultured cells  
 Preps in one run: 1 – 24  
 Macro name: mRNA\_L\_24

### Materials required

1. Samples in a 96 deep well plate, 1 ml
2. One sterile 96-well microplate, 300 µl
3. Reagent rack
4. RNase-free aerosol resistant Rainin 200 µl and 1000 µl tips in DiTi boxes
5. RNase-free PickPen® tips in 96 tip box

### Deck layout



## 7.10 MagRo™ 8-M purification from 10<sup>5</sup> – 10<sup>6</sup> cultured cells, 25 - 48 preps

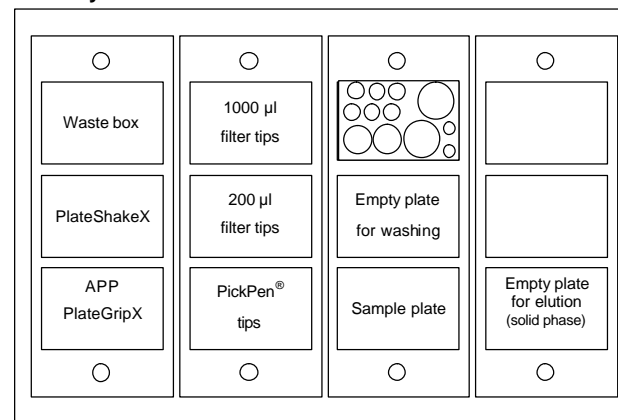
### Specifications

Sample material amount: 10<sup>5</sup> – 10<sup>6</sup> cultured cells  
 Preps in one run: 25 – 48  
 Macro name: mRNA\_L\_48

### Materials required

1. Samples in a 96 deep well plate, 1 ml
2. One sterile 96 deep well plate, 1 ml for washing
3. One sterile 96-well microplate, 300 µl for elution
4. Reagent rack
5. RNase-free aerosol resistant Rainin 250 µl and 1000 µl tips in DiTi boxes
6. RNase-free PickPen® tips in 96 tip box

### Deck layout



## 8 TROUBLESHOOTING GUIDE

### 8.1 Reagents

Low mRNA yield	
Poor sample homogenization	<p>Make sure that the sample is totally homogenized. Increase the homogenization time or try another homogenization method.</p> <p>Extrude the sample lysate through 19-G/21-G or 23G needle attached to syringe until sample is not viscous anymore</p> <p>If liquid nitrogen is used: Don't let the samples to thaw during or after homogenization Cut the sample into small pieces before homogenization</p> <p>Repeat purification with fresh sample</p>
Too small sample amount	<p>Try another homogenization method</p> <p>Use larger sample amounts or smaller reagent amounts (see Chapter 6 "Protocols for manual PickPen® 1-M and 8-M tools")</p>
Too large sample amount	Use smaller sample amount. Too high sample amount interfere with the purification. For larger sample amounts use more reagents (see Chapter 6 "Protocols for manual PickPen® 1-M and 8-M tools").
Insufficient binding	<p>Make sure the Magnetic Particles are in suspension during incubation</p> <p>Suspend Magnetic Particles gently by pipetting up and down before binding step</p> <p>Increase the binding time</p>
No shaking during incubations	Make sure the Magnetic Particles are in suspension during incubations
Insufficient washes	<p>Increase the washing time in each Wash Buffer</p> <p>Use the eluate as a sample and repeat the purification</p>
Inappropriate Elution Buffer	mRNA will only be eluted in the presence of low salt (e.g. 10 mM Tris-Cl, pH 7.5) or water. Check the pH and salt concentration of the Elution Buffer
Insufficient elution (optional)	Increase the elution time
Magnetic Particles	Ensure that elution is performed at +70°C
	Ensure that Magnetic Particles are in suspension during elution
	Optimize the amount of Magnetic Particles
	Use only mRNA Oligo (dT) <sub>30</sub> Magnetic Particles
	Do not freeze Magnetic Particles before purification
	Make sure that Magnetic Particles are uniformly suspended before dispensing

Purified mRNA too concentrated / too diluted	
Too small elution volume	Use more Elution Buffer to achieve optimal concentration
	Dilute final eluate by adding sufficient volume of Elution Buffer
Too large elution volume	Use less Elution Buffer to achieve optimal concentration

### 8.2 Manual Tools

Magnetic Particles are not collected from the suspension	
Magnet inside	Push the magnet out
No tip	Use PickPen® tip
Sample too viscous	<p>Make sure to use correct sample amounts and that homogenizing step is adequately performed</p> <p>Decrease the amount of sample material</p> <p>Dilute the sample</p> <p>Extrude the sample lysate through 19-G/21-G or 23G needle attached to syringe until sample is not viscous anymore</p>
Visible Magnetic Particles in all vessels/wells	Increase the collecting time and recollect Magnetic Particles
Visible Magnetic Particles after the optional elution step	<p>Centrifuge the sample for 1 minute with maximum speed</p> <p>Increase the collecting time</p>

Magnetic Particles are not released from the tip	
Magnet out	Pull the magnet inside
No tip	Use PickPen® tip
Sample amount too high	<p>Make sure to use correct sample amounts and that the homogenization are adequately performed</p> <p>Decrease the amount of sample material</p> <p>Dilute the sample using Lysis/Binding Buffer</p> <p>Increase the suspension time and rub the PickPen® tip with Magnetic Particles against the vessel wall</p>
Too small elution volume	Use larger volume

### 8.3 MagRo™ 8-M robotic workstation

MagRo™ 8-M does not work	
Instrument connections	<p>Check that the instrument is connected to the main power source and that all the cables are connected</p> <p>Check that the power is turned on in the back and in the front of the instrument</p>
Home-made process or macro does not start	<p>Check the process in Edit process or Edit macro</p> <p>Check that all the parameters are correctly written</p>

MagRo™ 8-M protocol problems	
No liquid aspirated	The tip might be blocked or broken. Do not reuse tips
	The pump piston may have some liquid inside, clean the pump
	Some reagents might have air bubbles that interfere with the aspiration.
"No liquid" signal	Check that reagent bottles contain liquids. Fill the bottles and continue the process.
The tips were not dropped in to waste box	Empty the waste box after each run
	Check that the waste slide is positioned at the right edge
Gripper malfunction	Check that the grippers are correctly placed on the holder
APP tool malfunction	<p>Check that the APP is correctly placed on the holder with no tips attached</p> <p>Make sure that there are enough tips for the APP</p>
Pump malfunction	Check that there are no tips attached to the pumps before run
Shaker	Make sure that there is enough tips for both pumps
	Check that the cables are connected
	Ensure the Heat Adapter is firmly placed on the shaker

	Use only new plates on the Heat Adapters (autoclaving may alter the plate dimensions) Check the shaking speed and maximum liquid volumes for each plate to prevent liquid spill. Check the temperature for each reagent and volume.
Labware	Place the plates in a correct orientation (A1 is in the upper left corner) Check the plate dimensions Use only new plates on the deck (autoclaving may alter the plate dimensions)
No tips taken	Ensure that the tip boxes are tightly in place Ensure that the tip boxes are filled Use only Rainin tips

#### 8.4 Downstream applications

Downstream applications	
DNA contamination	Treat the sample with DNase I Make sure to use correct sample amounts and that homogenizing step is adequately performed
No RT-PCR product	Extrude the sample lysate through 19-G / 21-G or 23G needle attached to syringe until sample is not viscous anymore Repeat purification with fresh sample
	Sequencing enzymes, polymerases and other Mg <sup>2+</sup> - dependent enzymes: EDTA inhibits enzymes, use water as elution buffer
	Optimize the mRNA amount for the application Wash the Magnetic Particles containing the bound mRNA properly in Wash Buffer A and B
	Perform a quick washing step with the 1 x downstream buffer, before suspending Magnetic Particles into Elution Buffer Use less mRNA-Magnetic Particle suspension in RT-PCR

#### 9 STORAGE AND STABILITY

The QuickPick™ SML mRNA purification kit should be stored at +2°C - +8°C. Magnetic Particles should not be frozen before mRNA purifications.

#### 10 WARNINGS AND LIMITATIONS

The QuickPick™ SML mRNA purification kit is intended for research use only, and is not intended for use in human diagnostic or therapeutic procedures. Standard methods for preventing contamination with RNases during preparation of mRNA must be taken. Precautions should also be taken to avoid contamination of opened vials. Do not pipet by mouth.

Lysis/Binding Buffer, Wash Buffer A and Wash Buffer B contain LiCl, and Lysis/Binding Buffer contains LiDS and DTT. Do not pipet by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions.

#### 11 DISCLAIMERS AND WARRANTIES

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