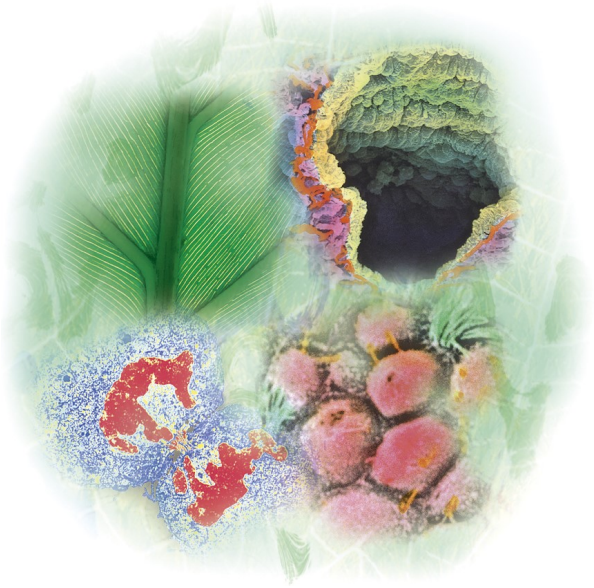
QuickPick™ SML mRNA

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



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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 QuicPick one magnet or QuicPick multiEight 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 QuicPick manual tools .

Principle of the method

QuickPick™ SML mRNA kits are based on the binding of poly-A(+) mRNA to Oligo $(dT)_{30}$ coated paramagnetic particles. Sample cells are lysed in the presence of Lysis/Binding Buffer. Cell lysate is incubated with Oligo $(dT)_{30}$ coated Magnetic Particles for hybridizing Oligo $(dT)_{30}$ 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.

SPECIFICATIONS

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

Sample	Amount	Amount	Amount
Cultured cells	≤ 10^3 cells	10^3 - 10^5 cells	10^5 - 10^6 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[1]	Applicable for RT-PCR		

[1]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.

KIT CONTENTS

Reagents for the SML kits

Reagent:	41002[1]	41012	41022
Oligo $(dT)_{30}$ coated Magnetic Particles	240 µl	0.80 ml	2 x 1.5 ml
Lysis/Binding Buffer[2,3,4]	1.6 ml	6 ml	22 ml
Wash Buffer A[2,3]	3.2 ml	12 ml	44 ml
Wash Buffer B[2]	1.6 ml	6 ml	22 ml
Elution Buffer	1.6 ml	6 ml	22 ml

[1]For manual use only.
[2]Reagent contains LiCl.
[3]Reagent contains LiDS.
[4]Reagent contains DTT.

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

Reagent:	Volume:	Product No:
QuickPick™ XL mRNA Oligo $(dT)_{30}$ coated Magnetic Particles	12.6 ml	41100
QuickPick™ XL mRNA Lysis/Binding Buffer[1,2,3]	83 ml	41300
QuickPick™ XL mRNA Wash Buffer A[1,2]	165 ml	41510
QuickPick™ XL mRNA Wash Buffer B[1]	83 ml	41520
QuickPick™ XL mRNA Elution Buffer	83 ml	41600

[1]Reagent contains LiCl.
[2]Reagent contains LiDS.
[3]Reagent contains DTT.

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 QuicPick tools”).

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	≤ 10^3 cells	10^3 - 10^5 cells	10^5 - 10^6 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
Sample Volume in Lysis/Binding Buffer	100 µl	200 µl	400 µl
Number of preps:			
41002[1]	16	8	4
41012	48	24	12
41022	192	96	48

[1]For manual use only.

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.

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”.

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 -80ºC, 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”.

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*).

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.

QUICPICK TIPS

The QuicPick tips packed in bulk quantities in plastic bags are not RNase-free. To eliminate RNase activity the tips should be washed in RNAse away or equivalent following the instructions provided by the manufacturer..

PROTOCOLS FOR MANUAL QUICPICK 1 AND multiEightTOOLS

QuicPick 1 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 $(dT)_{30}$ 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 (+70ºC) 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 +70ºC for 5 minutes. While poly-A(+) mRNA is eluted from the Magnetic Particles the QuicPickTip may be detached from the QuicPick 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 QuicPick 1 purifications.

Reagent	**Reagent volume per preparation**		
Sample amount[1]	**≤ 10^3 cells**	**10^3 - 10^5 cells**	**10^5 - 10^6 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

[1] 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. QuicPick tool and RNase-free 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:

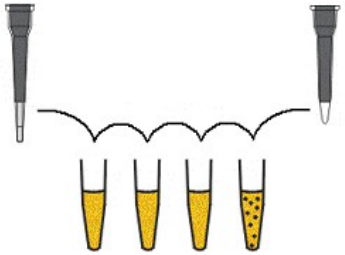
Tube 1: Sample in Lysis/Binding Buffer
and Oligo $(dT)_{30}$ coated Magnetic Particles
Tube 2: Wash Buffer A
Tube 3: Wash Buffer A
Tube 4: Wash Buffer B
Tube 5: Elution Buffer

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

3. 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

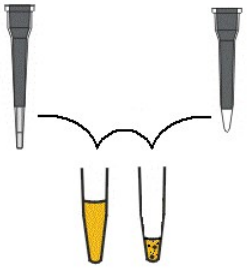
4. Pick up the QuicPick tip with the QuicPick 1. Collect the Magnetic Particles from tube 1 with QuicPick 1 and release them into tube 2 (Wash Buffer A). Wash the Magnetic Particles by mixing the suspension gently for 5 - 15 seconds using the QuicPick tip. Note that the magnet has to be withdrawn at this point. Repeat the washing steps in tubes 3 and 4 (Wash Buffer A and Wash Buffer B).



1. 2. 3. 4.

Washing steps

5. Collect the Magnetic Particles from tube 4 with QuicPick 1 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.



4. 5.

Solid phase elution

QuicPick multiEight 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 $(dT)_{30}$ coated Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended.
3. Repeat or 8-channel pipettors should not be used when dispensing Magnetic Particles.
4. 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.
5. RNase-free water can also be used for elution.
6. Maintain RNase-free conditions while working with RNA.
7. β-mercaptoethanol can be used in Lysis/Binding Buffer for inhibition of RNases. Add 10 µl β-mercaptoethanol in 1 ml Lysis/Binding Buffer.
8. 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.
9. 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.
10. 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.
11. 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 QuicPick multiEight purifications.

Reagent	**Reagent volume per preparation**		
Sample amount[1]	**≤ 10^3 cells**	**10^3 - 10^5 cells**	**10^5 - 10^6 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

[1]Cultured cells used as an example, for the amounts of other sample materials, see Table 2.

Materials required

1. Sterile U-bottom 96-well plates (see Table 5).
2. Pipettes and RNase-free aerosol resistant micropipettor tips.
3. QuicPick multiEight tool and Rnase-free QuicPick tips in tip box.
4. Orbital shaker for 96-well plates.

5. Optional: Syringes and needles (for homogenization viscous cell lysates).
6. 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[1]	Recommended plate	Bio-Nobile Product No.
≤ 10^3 cells	Nunc 96-well microplate, 300 µl Sterile	M1-262126, 10 plates M-262162, 50 / case
10^3 – 10^5 cells	Nunc 96-well microplate, 500 µl Sterile	M1-267334, 10 plates M-267334, 120 / case
10^5 – 10^6 cells	Nunc 96 deep-well, 1 ml Sterile	M1-260251, 10 plates M-260251, 50 / case

[1] Cultured cells used as an example, for the amounts of other sample materials, see Table 2.

Protocol

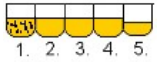
The following instructions are for 8 samples. Samples are prepared in tubes and transferred into 96-well plates (U-bottom) where the rest of the protocol is carried out. The optional elution step can also be performed in a heating block or an equivalent device suitable for 96-well plates.

1. Prepare the samples (number tubes from 1 to 8) according to Chapter 4 “Sample preparation”.
2. 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 $(dT)_{30}$ coated Magnetic Particles
   Column 2: Wash Buffer A
   Column 3: Wash Buffer A
   Column 4: Wash Buffer B
   Column 5: Elution Buffer

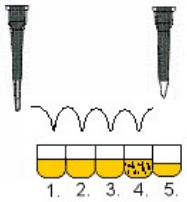
All reagents and the samples should be kept at room temperature during the mRNA purification procedure.

3. 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.

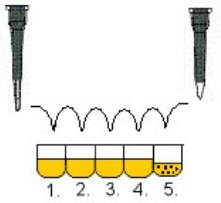


Incubate for 5 minutes at room temperature with continuous mixing

Pick up the QuicPick tips using QuicPick multiEight. Collect the Magnetic Particles from column 1 with QuicPick tool and release them into column 2 (Wash Buffer A). Mix the suspensions gently for 5 – 15 seconds using the QuicPick 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 QuicPick multiEight 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 -80ºC for later use.



Solid phase elution

TROUBLESHOOTING GUIDE

Reagents

Low mRNA yield	
Poor sample homogenization	Make sure that the sample is totally homogenized. Increase the homogenization time or try another homogenization method.
	Extrude the sample lysate trough 19-G/21-G or 23G needle attached to syringe until sample is not viscous anymore
	If liquid nitrogen is used: Don't let the samples to thaw during or after homogenization
	Cut the sample into small pieces before homogenization
	Repeat purification with fresh sample
	Try another homogenization method
Too small sample amount	Use larger sample amounts or smaller reagent amounts
Too large sample amount	Use smaller sample amount. Too high sample amount interfere with the purification. For larger sample amounts use more reagents
Insufficient binding	Make sure the Magnetic Particles are in suspension during incubation
	Suspend Magnetic Particles gently by pipetting up and down before binding step
	Increase the binding time
No shaking during incubations	Make sure the Magnetic Particles are in suspension during incubations
Insufficient washes	Increase the washing time in each Wash Buffer
	Use the eluate as a sample and repeat the purification
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
	Ensure that elution is performed at +70°C
	Ensure that Magnetic Particles are in suspension during elution
Magnetic Particles	Optimize the amount of Magnetic Particles
	Use only mRNA Oligo $(dT)_{30}$ Magnetic Particles
	Do not freeze Magnetic Particles before purification
	Make sure that Magnetic Particles are uniformly suspensed 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

Manual Tools

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

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

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
	Extrude the sample lysate trough 19-G / 21-G or 23G needle attached to syringe until sample is not viscous anymore
No RT-PCR product	Repeat purification with fresh sample
	Sequencing enzymes, polymerases and other Mg^{2+} - 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 suspensing Magnetic Particles into Elution Buffer
	Use less mRNA-Magnetic Particle suspension in RT-PCR

STORAGE AND STABILITY

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

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 pipette by mouth.

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

DISCLAIMERS AND WARRANTIES

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