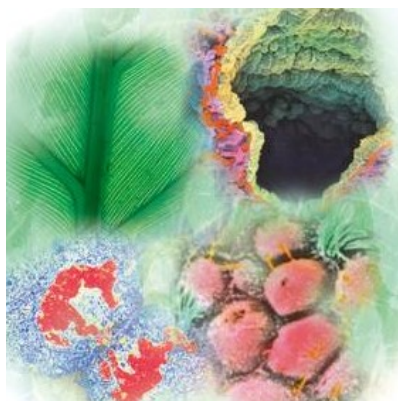


QuickPick™ SML total RNA

- 42002 • total RNA purification kit, 8 preps
- 42012 • total RNA purification kit, 24 preps
- 42022 • total RNA purification kit, 96 preps



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INTRODUCTION

These are the instructions for use for the QuickPick™ SML total RNA purification kits. Please read the entire instructions carefully before starting the work. Also refer to the QuickPick instructions for use.

QuickPick™ SML total RNA purification kits provide a fast and simple means of purifying total RNA from whole blood, tissues 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 total RNA can be used for downstream applications such as RT-PCR.

The reagent volumes can be scaled up or down to be used for different sample amounts either with the QuickPick manual tools .

Principle of the method

RNA in the sample is released using Proteinase K solution and Lysis Buffer. Released RNA is bound to Magnetic Particles in the presence of Binding Buffer. To prevent the co-purification of interfering DNA, a DNase I treatment is performed after the first wash step. Magnetic Particles with the bound RNA are finally washed with Wash Buffer and then the RNA is eluted from the Magnetic Particles with the Elution Buffer.

SPECIFICATIONS

Table 1: Specifications for QuickPick™ SML total RNA purification kits.

Sample	Amount	Yield	Amount	Yield	Amount	Yield
Whole Blood ⁽¹⁾	50 µl	Up to 0.5 µg	100 µl	Up to 1.0 µg		
Plant tissue (<i>Hordeum vulgare</i>) Tissue (liver)	25 mg	Up to 3 µg	50 mg	Up to 6 µg		
	2.5 mg	Up to 3 µg	5.0 mg	Up to 6 µg	10.0 mg	12 µg
Typical purity ⁽²⁾	≥ 1.9					
Downstream suitability	Applicable for RT-PCR					

¹The yield is highly donor-dependent, thus, higher or lower values may be obtained.

²Ratio of absorbance at 260/280 nm is corrected with absorbance at 320 nm.

KIT CONTENTS

Reagents for the SML kits

Reagent:	42002	42012	42022
Total RNA MagaZorb Magnetic Particles ^(1,2)	80 µl	240 µl	960 µl
Total RNA Proteinase K solution	80 µl	240 µl	960 µl
Total RNA Lysis Buffer	800 µl	2.4 ml	9.6 ml
Total RNA Binding Buffer ⁽²⁾	2 ml	6 ml	24 ml
Total RNA Wash Buffer ⁽²⁾	8 ml	24 ml	2 x 48 ml
Total RNA DNase Buffer ^(2,3)	800 µl	2.4 ml	9.6 ml
Total RNA Elution Buffer	400 µl	1.2 ml	4.8 ml

¹MagaZorb technology is a trademark of Cortex Biochem Inc.

²Contains 0.02 % NaN₃.

³DNase I is not included in the kit.

Scaling of sample amounts

The sample amount can be scaled for 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.

Table 2: The effect of sample amount on number of preparations for some purifications with QuickPick™ SML total RNA kit. (The reference volumes are shown in bold.)

Sample	Amount of sample per preparation			
Whole Blood	25 µl	50 µl	100 µl	
Plant tissue	12.5 mg	25 mg	50 mg	
Tissue	1.25 mg	2.5 mg	5.0 mg	10.0 mg
Number of preps:				
42002	32	16	8	4
42012	96	48	24	12
42022	384	192	96	48

SAMPLE PREPARATION

The user should utilize a sample preparation method that is known to yield undegraded RNA. Maintain RNase-free conditions during the sample preparation.

Sample preparation from blood

Human whole blood

Total RNA can be purified from fresh human whole blood which has been treated with commonly used anticoagulants; EDTA, heparin or citrate. Blood samples should not be frozen.

Sample preparation from plant tissue

Complete disruption of cell walls is essential to release all the nucleic acids from the tissue. Insufficient disruption of starting material will lead to low RNA 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.

It is preferable to harvest young plant material (e.g. expanding leaves or needles). RNA 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 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 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 the frozen sample in a 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 appropriate volume of total RNA Lysis Buffer and Proteinase K solution (Tables 3 and 4) before the sample thaws.
9. Proceed with the purification protocol.

Homogenization using tissue grinder

A tissue grinder homogenizes plant tissue samples efficiently and helps in rapid preparation of the sample homogenates. When using fresh plant leaf tissues, most samples can be homogenized in the presence of Lysis Buffer. Alternatively, homogenization of frozen plant materials can be performed without Lysis Buffer if the disruption vessel is precooled with liquid nitrogen. Plant tissue samples should be homogenized in the presence of either Lysis Buffer or liquid nitrogen to preserve the quality of the contained nucleic acids.

1. Weigh the plant tissue samples into tubes.
2. Add liquid nitrogen into the tubes, but be careful not to spill the samples out of the tubes.
3. Homogenize the plant tissue samples for 1 - 2 minutes with the tissue grinder (for example Pellet Pestle or equivalent device) until fine whitish powder results.
4. Ensure all liquid nitrogen has evaporated before closing the tubes (Do not let the samples thaw).
5. If the samples are not processed immediately, the tubes should be kept on dry ice or liquid nitrogen or stored at -80°C, to prevent the samples from thawing after evaporation.
6. If the samples are processed immediately after homogenization, add appropriate volumes of total RNA Lysis Buffer and Proteinase K solution (Tables 3 and 4) before the samples thaw.
7. Proceed with the purification protocol.

Sample preparation from tissue

Complete disruption of cell walls, plasma membranes, and organelle membranes is essential to release all the nucleic acids contained in the tissue. Insufficient disruption of starting material will lead to low RNA yield. Cell wall properties vary widely between species and proper homogenization method should be applied to achieve complete disruption. The homogenization can be performed for example by mechanical grinding. Other homogenization methods can also be used.

Keep tissue sample on ice during the sample preparation. Homogenize the tissue with a tissue grinder (Pellet Pestle or equivalent device).

Homogenization using tissue grinder

Tissue grinders disrupt samples efficiently and help in rapid preparation of the sample homogenate.

1. Weigh the tissue samples into 2 ml tubes.
2. Add appropriate volume of RNA_{later} (100 µl RNA_{later} / 5.0 mg tissue) into the tubes.
3. Homogenize the tissue samples manually or with a Pellet Pestle or equivalent device. A homogeneous suspension should be obtained within 5 minutes.
4. Add appropriate volumes of total RNA Lysis Buffer and Proteinase K solution (Tables 3 and 4).
5. Proceed with the purification protocol.

Note: RNA_{later} or RNase Away are not included in the kit.

QUICK PICK TIPS

Eliminating RNases from QuickPick tips

For QuickPick tips, Place QuickPick tips in glass beaker so that the heads of the tips are towards the bottom of the beaker. Treat with RNase solution according to instruction. Dry in an oven at 50°C.

PIPETTING TIPS

It is recommended to use aerosol resistant tips or filter tips for liquid dispensing throughout the process.

PROTOCOLS FOR MANUAL QuicPick one magnet and QuicPick multiEight TOOLS

QuicPick one magnet protocols

Notes

1. All solutions should be clear when used. If precipitates have formed, warm the solutions gently until the precipitates have dissolved.
2. Total RNA MagaZorb Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended. Repeat pipettors should not be used when dispensing Magnetic Particles
3. DNase I should be added to the Total RNA DNase Buffer prior the purification to a final concentration of 40 U/ml. The mixture should be prepared on the same day and can not be stored.
4. Lysis time can be increased if using difficult samples.
5. Water can also be used for elution.
6. For purifications with 10 mg of tissue use 2 ml tubes during the purification.
7. Maintain RNase-free conditions while working with RNA. It is recommended to keep the caps of the tubes and reagent bottles open only the time that is needed for operations.
8. During the incubation steps the QuicPick tip can be stored in a clean RNase-free tube, if needed.

Reagent volumes

Table 3: Reagent volumes for QuicPick 1 magnet purifications.

Reagent	Reagent volume per preparation			
Sample amount ⁽¹⁾	25 µl	50 µl	100 µl	10 mg
Proteinase K solution	2.5 µl	5 µl	10 µl	20 µl
Lysis Buffer	25 µl	50 µl	100 µl	200 µl
Binding Buffer	62.5 µl	125 µl	250 µl	500 µl
Magnetic Particles	2.5 µl	5 µl	10 µl	20 µl
Wash Buffer	2 x 125 µl	2 x 250 µl	2 x 500 µl	2 x 1000 µl
DNase Buffer ⁽²⁾	25 µl	50 µl	100 µl	200 µl
Elution Buffer	5 - 25 µl	10 - 25 µl	25 - 50 µl	50 - 100 µl

¹Whole blood 25 – 100 µl and tissue 10 mg used as an example. The corresponding amounts of plant material and tissue (see Table 2) may also be used as the sample.

²Add DNase I into DNase Buffer prior the purification as described in Note 3 in Chapter 7.1 “QuicPick Protocol”. **DNase I is not included in the kit.**

Materials required

1. Sterile RNase-free 1.5 – 2.0 ml tubes.
2. Pipettes and sterile RNase-free aerosol resistant micropipettor tips.
3. QuicPick 1 magnet tool and RNase-free tips.
4. DNase 1

Protocol for whole blood

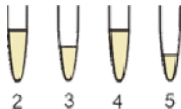
1. Number five tubes from 1 to 5.
2. Pipette appropriate volumes of Proteinase K solution, sample and Lysis Buffer into tube 1 according to the Table 3. Mix well by inverting the tube several times and pulse-vortexing for 15 seconds. Lyse the sample at 56°C for 10 minutes.



Incubate for 10 minutes at 56°C

3. During the lysis step, pipette QuicPick™ SML total RNA purification kit reagents (according to the Table 3) into tubes 2 - 5 as follows:

- Tube 2: Total RNA Wash Buffer
 Tube 3: Total RNA DNase Buffer (DNase I added)
 Tube 4: Total RNA Wash Buffer
 Tube 5: Total RNA Elution Buffer



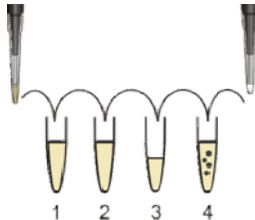
4. Remove tube 1 from the incubation at 56°C. Pipette appropriate volumes of total RNA Binding Buffer and total RNA MagaZorb Magnetic Particles into tube 1 according to the Table 3.

5. Mix tube 1 gently and incubate at room temperature for 5 minutes. Mix the suspension continuously during this step. (Use a tube rotator or mix manually). Normally, the Magnetic Particles form a lump when RNA/DNA binds to them.



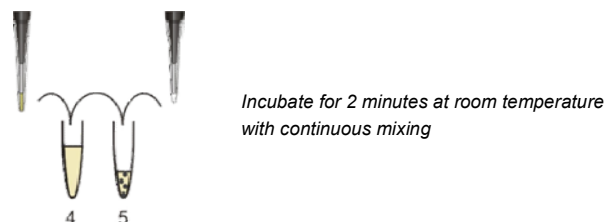
Incubate for 5 minutes at room temperature
with continuous mixing

6. Pick up the QuicPick tip with the tool. Collect the Magnetic Particles from tube 1 with QuicPick and release them into tube 2 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the tip. Note that the magnet has to be withdrawn at this point.

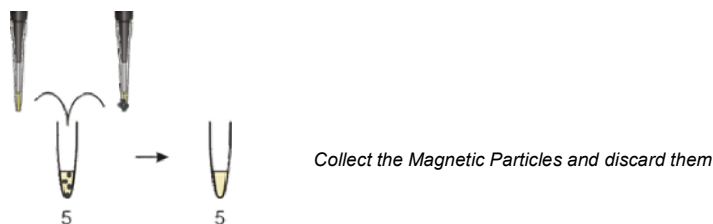


Washing and DNA degradation steps

- Collect the Magnetic Particles from tube 2 with QuicPick and release them into tube 3 (DNase Buffer). Mix tube 3 continuously, and incubate at room temperature for 5 minutes (use a tube rotator or mix manually). The co-purified DNA is degraded during this step. During DNase treatment the lump of Magnetic Particles should disperse completely.
- Collect the Magnetic Particles from tube 3 with QuicPick and release them into tube 4 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the tip. Note that the magnet has to be withdrawn at this point.
- Collect the Magnetic Particles from tube 4 with QuicPick and release them into tube 5 (Elution Buffer). Mix tube 5 continuously, and incubate at room temperature for 2 minutes (use a tube rotator or mix manually).

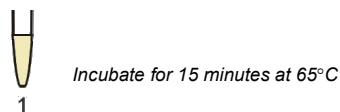


- Collect the Magnetic Particles from tube 5 and discard them and the tip. The eluate in tube 5 containing the purified RNA is ready to be used in downstream applications. If the purified RNA is not used on the same day, store at -80°C until use.



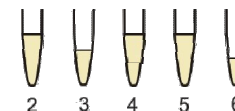
Protocol for plant tissue

- Number six tubes from 1 to 6.
- Prepare the plant tissue sample according to Chapter 4 "Sample preparation". Add appropriate volumes of Lysis Buffer and Proteinase K solution into the plant tissue sample (see Table 3). Mix thoroughly by pulse-vortexing and lyse the sample for 15 minutes at 65°C.

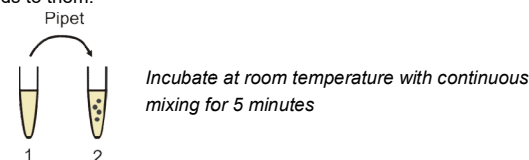


- During the lysis step, pipette QuicPick™ SML total RNA reagents (according to the Table 3) into tubes 2 - 6 as follows:

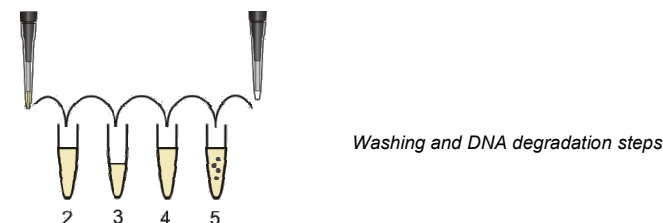
Tube 2:	Total RNA MagaZorb Magnetic Particles and total RNA Binding Buffer
Tube 3:	Total RNA Wash Buffer
Tube 4:	Total RNA DNase Buffer (DNase I added)
Tube 5:	Total RNA Wash Buffer
Tube 6:	Total RNA Elution Buffer



- Remove tube 1 from incubation at 65°C. Centrifuge the tube for 5 minutes at 18,000 x g. Gently transfer the supernatant into tube 2 (Binding Buffer, Magnetic Particles). Mix tube 2 gently and incubate at room temperature for 5 minutes. Mix the suspension continuously during this step (use a tube rotator or mix manually). Normally, the Magnetic Particles form a lump when RNA/DNA binds to them.

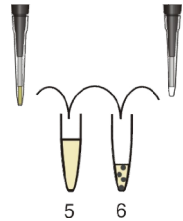


- Pick up the QuicPick tip with the tool. Collect the Magnetic Particles from tube 2 with QuicPick and release them into tube 3 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the tip. Note that the magnet has to be withdrawn at this point.



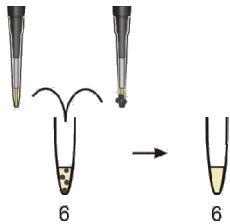
- Collect the Magnetic Particles from tube 3 with QuicPick and release them into tube 4 (DNase Buffer). Mix tube 4 continuously, and incubate at room temperature for 5 minutes (use a tube rotator or mix manually). The co-purified DNA is degraded during this step. During DNase treatment, the lump of Magnetic Particles should disperse completely.
- Collect the Magnetic Particles from tube 4 with QuicPick and release them into tube 5 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the tip. Note that the magnet has to be withdrawn at this point.

8. Collect the Magnetic Particles from tube 5 with QuicPick and release them into tube 6 (Elution Buffer). Mix tube 5 continuously, and incubate at room temperature for 2 minutes (use a tube rotator or mix manually).



Incubate for 2 minutes at room temperature with continuous mixing

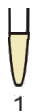
9. Collect the Magnetic Particles from tube 6 and discard them and the tip. The eluate in tube 6 containing the purified RNA is ready to be used in downstream applications. If the purified RNA is not used on the same day, store at -80°C until use.



Collect the Magnetic Particles and discard them

Protocol for tissue

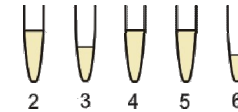
1. Number six tubes from 1 to 6.
2. Prepare the tissue sample according to Chapter 4 "Sample preparation". Add appropriate volumes of Lysis Buffer and Proteinase K solution into the tissue sample (see Table 3). Mix thoroughly by pulse-vortexing and lyse the sample for 10 minutes at 56°C.



Incubate for 10 minutes at 56°C

3. During the lysis step, pipette QuicPick™ SML total RNA reagents (according to the Table 3) into tubes 2 - 6 as follows:

Tube 2: Total RNA MagaZorb Magnetic Particles and total RNA Binding Buffer
 Tube 3: Total RNA Wash Buffer
 Tube 4: Total RNA DNase Buffer (DNase 1 added)
 Tube 5: Total RNA Wash Buffer
 Tube 6: Total RNA Elution Buffer

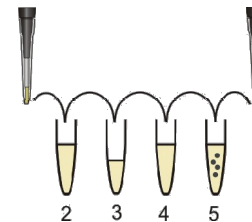


4. Remove tube 1 from incubation at 65°C. Centrifuge the tube for 5 minutes at 18,000 x g. Gently transfer the supernatant into tube 2 (Binding Buffer, Magnetic Particles). Mix tube 2 gently and incubate at room temperature for 5 minutes. Mix the suspension continuously during this step. (Use a tube rotator or mix manually). Normally, the Magnetic Particles form a lump when RNA/DNA binds to them.



Incubate at room temperature with continuous mixing for 5 minutes

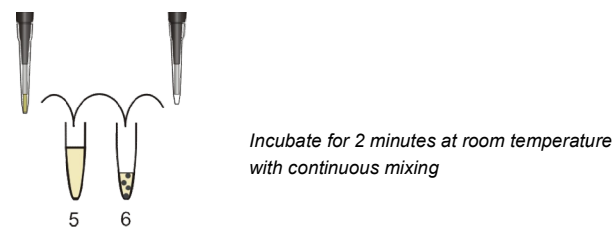
5. Pick up the QuicPick tip with the tool. Collect the Magnetic Particles from tube 2 with QuicPick and release them into tube 3 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the tip. Note that the magnet has to be withdrawn at this point.



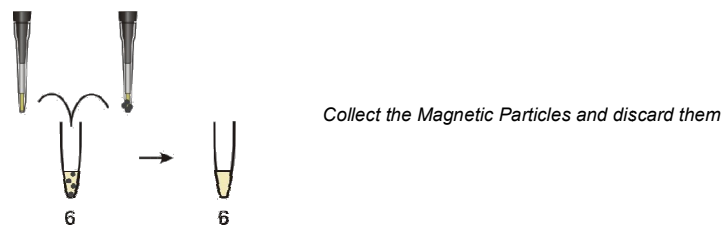
Washing and DNA degradation steps

5. Collect the Magnetic Particles from tube 3 with QuicPick 1 and release them into tube 4 (DNase Buffer). Mix tube 4 continuously, and incubate at room temperature for 5 minutes (use a tube rotator or mix manually). The co-purified DNA is degraded during this step. During DNase treatment the lump of Magnetic Particles should disperse completely.
6. Collect the Magnetic Particles from tube 4 with QuicPick 1 and release them into tube 5 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the QuicPick tip. Note that the magnet has to be withdrawn at this point.

7. Collect the Magnetic Particles from tube 5 with QuicPick 1 and release them into tube 6 (Elution Buffer). Mix tube 6 continuously, and incubate at room temperature for 2 minutes (use a tube rotator or mix manually).



8. Collect the Magnetic Particles from tube 6 and discard them and the tip. The eluate in tube 6 containing the purified RNA is ready to be used in downstream applications. If the purified RNA is not used on the same day, store at -80°C until use.



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. Total RNA MagaZorb 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. DNase I (**NOT PROVIDED IN KIT**) should be added to the Total RNA DNase Buffer prior the purification to a final concentration of 40 U/ml. The mixture should be prepared on the same day and can not be stored.
5. 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 the Magnetic Particles in suspension.
6. Lysis time can be increased with difficult samples.
7. Water can also be used for elution.
8. Maintain RNase-free conditions while working with RNA. It is recommended to keep the reagent bottles open only the time that is needed for operations.

Reagent volumes

Table 4: Reagent volumes for QuicPick multiEight purifications.

Reagent	Reagent volume per preparation		
Sample amount ⁽¹⁾	25 µl	50 µl	100 µl
Lysis Buffer	25 µl	50 µl	100 µl
Proteinase K solution	2.5 µl	5 µl	10 µl
Binding Buffer	62.5 µl	125 µl	250 µl
Magnetic Particles	2.5 µl	5 µl	10 µl
Wash Buffer	2 x 125 µl	2 x 250 µl	2 x 500 µl
DNase Buffer ⁽²⁾	25 µl	50 µl	100 µl
Elution Buffer	5 - 25 µl	10 - 25 µl	25 - 50 µl

¹Whole blood used as an example. The corresponding amounts of plant material and tissue (see Table 2) may also be used as the sample.

²Add DNase I into DNase Buffer prior the purification as described in Note 4 in Chapter 7.2 “Quicpick multiEight Protocol”. **DNase I is not included in the kit.**

Materials required

1. Sterile U-bottom 96-well plates (for example, Nunc 500 µl 96-well microplate or 1 ml x 96 deep-well plate.
2. Pipettes and sterile RNase-free micropipettor tips.
3. QuicPick multiEight tool and RNase-free QuicPick tips.
4. Orbital shaker for 96-well plates.

Protocol for whole blood

Protocols for other sample materials are available upon request.

The following instructions are for 8 samples. Samples are lysed in tubes and transferred into 96-well plates (U-bottom) where the rest of the protocol is carried out. The lysis step can also be performed in a thermal shaker using suitable adapter for 96-well plate.

1. Prepare the samples by pipetting appropriate volumes of Proteinase K solution, sample and Lysis Buffer into tubes 1-8 according to the Table 4. Mix the tubes well by inverting each tube several times and pulse-vortexing for 15 seconds. Lyse samples at 56°C for 10 minutes.



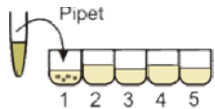
Incubate for 10 minutes at 56°C

2. During the lysis step, pipette QuickPick™ SML total RNA purification kit reagents (according to the Table 4) into 96-well plate columns 1 - 5 as follows:

Column 1: Total RNA Magnetic Particles and Total RNA Binding Buffer
 Column 2: Total RNA Wash Buffer
 Column 3: Total RNA DNase Buffer (DNase I added)
 Column 4: Total RNA Wash Buffer
 Column 5: Total RNA Elution Buffer

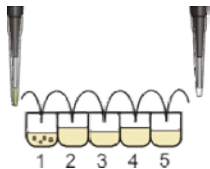


3. Remove the tubes from incubation at 56°C and spin shortly. Gently transfer the whole suspension from each tube into the respective wells of column 1 (Binding Buffer, Magnetic Particles) by mixing the lysed sample and Magnetic Particles carefully by pipetting up and down a few times. Mix the 96-well plate on the orbital shaker for 5 minutes at room temperature. Make sure that the Magnetic Particles are in suspension during this step. Normally, the Magnetic Particles form a lump when RNA/DNA binds to them.



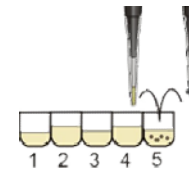
*Incubate for 5 minutes at room temperature
with continuous mixing*

Pick up the QuickPick tips using QuickPick multiEight . Collect the Magnetic Particles from column 1 with QuickPick multiEight and release them into column 2 (Wash Buffer). Mix the suspensions gently for 10 – 20 seconds using the QuickPick tips. Note that the magnets have to be withdrawn at this point.



Washing and DNA degradation steps

4. Collect the Magnetic Particles from column 2 with QuickPick tool and release them into column 3 (DNase Buffer). Mix the 96-well plate on the orbital shaker for 5 minutes at room temperature. The co-purified DNA is degraded during this step. During DNase treatment the lump of Magnetic Particles should disperse completely.
5. Collect the Magnetic Particles from column 3 with QuickPick and release them into column 4 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the QuickPick tip. Note that the magnets have to be withdrawn at this point.
6. Collect the Magnetic Particles from column 4 with QuickPick 8-M and release them into column 5 (Elution Buffer). Mix the 96-well plate on the orbital shaker for 2 minutes at room temperature. Make sure that the Magnetic Particles are in a suspension during this step.
7. Collect the Magnetic Particles from column 5 and discard them and the tip. The eluates in column 5 contain the purified RNA and are ready to be used in downstream applications. If the purified RNA is not used on the same day, store at -80°C until use.



*Collect the Magnetic Particles
and discard them*

TROUBLESHOOTING GUIDE

Reagents

Low RNA yield	
RNase contamination	Decontaminate working area. Wipe all surfaces with RNase decontamination reagent. Wear protective clothes and gloves. Use filter-tips, RNase-free QuicPick tips and baked glassware.
Poor sample preparation	Use only fresh blood. Repeat purification with fresh sample Try another homogenization method for tissue or plant tissue samples
Too small sample amount	Use larger sample amount or smaller reagent volumes
Too large sample amount	Use smaller sample amount or diluted sample. Too high sample amount interfere with the purification. For larger sample amounts use more reagents.
Insufficient lysis	Add correct volume or increase the volume of Proteinase K solution and Lysis Buffer
	Use increased lysis time and/or improve mixing
	Make sure to mix sample, Proteinase K solution and Lysis Buffer thoroughly by inverting tube several times and pulse vortexing for 15 seconds before starting the lysis incubation. Also during the lysis the solution may be pulse-vortexed occasionally.
	Ensure that the heating step is done at 56°C or at 65°C
Insufficient binding	Make sure the Magnetic Particles are in suspension during incubations
	Suspend Magnetic Particles gently by pipetting up and down before binding step
	Increase the binding time
	Make sure the volume of the Binding Buffer correlates with the sample amount used
No shaking during incubations	Make sure that Magnetic Particles are in suspension during incubations
Insufficient elution	Increase the elution time Ensure that the Magnetic Particles are in suspension during elution Use heating during elution (max +65°C)
Magnetic particles	Optimize Magnetic Particle amount
	Use only total RNA MagaZorb Magnetic Particles
	Do not freeze Magnetic Particles
	Make sure that Magnetic Particles are uniformly suspended before dispensing

Purified RNA too concentrated / too diluted

Too small elution volume	Use more Elution Buffer to achieve optimal concentration Dilute final eluate by adding sufficient amount of Elution Buffer
Too large elution volume	Use less Elution Buffer to achieve optimal concentration

Insufficient DNase treatment

DNA present in elution buffer	Increase the incubation time Use higher concentration of DNase I
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Manual Tools

Magnetic Particles are not collected from the suspension

Magnet inside	Push the magnet out
Sample too viscous	Make sure to use correct sample amounts and that the homogenization and the lysis steps are adequately performed Decrease the amount of sample material Dilute the sample and use Lysis Buffer, Proteinase K solution and Binding Buffer in correct ratio
Visible Magnetic Particles in all vessels/wells	Repeat the collections
Visible Magnetic Particles in Elution Buffer	Centrifuge the sample for 1 min with maximum speed Increase the collection time

Magnetic Particles are not released from the tip

Magnet out	Pull the magnet inside
No tip	Use QuicPick tip
Sample amount too high	Make sure to use correct sample amounts and that the homogenization and the lysis steps are adequately performed Decrease the amount of sample material Dilute the sample and use Lysis Buffer, Proteinase K solution and Binding Buffer in correct ratio Increase 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	
High absorbance at 320 nm	Eluates may contain Magnetic Particles. Centrifuge the sample for 1 min with maximum speed. Wash the Magnetic Particles with bound RNA properly in Wash Buffers
No PCR product	Repeat the purification process using fresh sample Sequencing enzymes, polymerases and other Mg ²⁺ - dependent enzymes: EDTA inhibits the enzymes, use water as elution buffer Optimize the RNA amount for the application Wash the Magnetic Particles containing the bound RNA properly in Wash Buffers

STORAGE AND STABILITY

The QuickPick™ SML total RNA purification kit should be stored at room temperature. Magnetic Particles should not be frozen.

WARNINGS AND LIMITATIONS

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

Total RNA MagaZorb reagent, Wash Buffer, DNase Buffer and Binding Buffer contain 0.02 % sodium azide (NaN_3) as a preservative. When in contact with acid or heavy metal ions, it forms a highly toxic gas. Preservatives such as NaN_3 are toxic if ingested. Do not pipet by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions.

DISCLAIMERS AND WARRANTIES

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