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## 2. Spin Smart RNA Kit Components

<b>Kit Contents</b>	<b>50 preps</b>	<b>250 preps</b>
RNA 1 Lysis Buffer	25 ml	125 ml
RNA 3 Wash Buffer	15 ml	80 ml
RNA 4 Wash Buffer	12.5 ml	3 × 25 ml
RNA 2 Wash Buffer	25 ml	125 ml
Reaction Buffer for rDNase	7 ml	35 ml
rDNase	1 vial	5 vials
RNase-Free H <sub>2</sub> O	15 ml	65 ml
SpinSmart Filter Columns (purple ring)	50	250
SpinSmart RNA Binding Columns (Blue)	50	250
Collection Tubes (2 ml)	150	750
Collection Tubes (1.5 ml)	50	250
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### 3. The Spin Smart RNA Purification Procedure

The SpinSmart RNA purification technologies utilize a lysis buffer containing chaotropic salts, which effectively inactivates RNases. Further, it creates conditions which allow RNA to bind to the SpinSmart silica membrane. An on-column rDNase digest removes contaminating DNA, which also binds to the column. Subsequent washes with our buffers remove salts and cellular debris. Highly pure RNA is eluted with RNase-free Water, typical  $A_{260/280}$  ratio of 1.9 or above (absorbance measured in TE buffer, pH 7.5).

Spin Smart RNA kits are designed for use at room temperature. Eluted RNA should be frozen immediately at  $-20^{\circ}\text{C}$  (short-term storage) or  $-70^{\circ}\text{C}$  (long-term storage).

Spin Smart RNA purification kits are designed to isolate total RNA from cultured cells and tissue. The purified RNA is ready to use for typical molecular applications: reverse transcriptase-PCR\* (RT-PCR\*), primer extension, or RNase protection assays.

Average yield is between 5  $\mu\text{g}$  -70  $\mu\text{g}$  total RNA, depending on sample.

Average yields of total RNA isolation using SpinSmart RNA Purification kit	
Sample	average yield ( $\mu\text{g}$ )
$8 \times 10^4$ HeLa cells	1.5
$4 \times 10^5$ HeLa cells	4
$1 \times 10^6$ HeLa cells	14
$2 \times 10^6$ HeLa cells	21
$2.5 \times 10^6$ HeLa cells	25
$5 \times 10^6$ HeLa cells	50

SpinSmart RNA Purification Specifications

<b>Sample size</b>	< 5 x 10 <sup>6</sup> cells < 30 mg tissue
<b>Average yield</b>	up to 70 µg
<b>Elution volume</b>	40 - 120 µl
<b>Binding capacity</b>	100 µg
Time/prep	30 min for 6 preps
Spin column type	mini

Recommendations for Different Sample Types

Sample	Amount	Buffer Volumes	
		RNA 1 Lysis Buffer	Ethanol
Cultured animal or human cells (e.g. HeLa cells)	up to 5 x 10 <sup>6</sup>	350 µl	350 µl
Human or animal tissue	up to 20 mg 20 - 30 mg	350 µl *600 µl	350 µl *600 µl
Tissue stored in RNA <sup>later</sup> <sup>®</sup>	up to 20 mg 20 - 30 mg	350 µl *600 µl	350 µl *600 µl
Difficult to lyse tissue samples	up to 30 mg	*600 µl	*600 µl

\*If 600 µl RNA 1 Lysis Buffer and ethanol are used, sample must be loaded onto the column in two successive centrifugation steps.

### 3.1. Preparation And Storage Of Starting Materials

RNA is exposed to RNA-degrading enzymatic activity until the sample material is flash frozen or disrupted using RNase-inhibiting agents. Samples should be flash frozen in liquid N<sub>2</sub> immediately and stored at -70°C or processed as soon as possible. Samples can be stored in RNA 1 Lysis Buffer after disruption at -70°C for up to one year or up to several hours at room temperature. Frozen samples are stable up to 6 months. Samples frozen in RNA 1 Lysis Buffer should be thawed slowly before starting with RNA isolation procedure.

Wear gloves at all times during the preparation. Change gloves frequently to avoid contaminating samples with RNases.

#### Cultured Cells

Cultured cells are collected by centrifugation and directly lysed by adding RNA 1 Lysis Buffer according to step 2 of the standard protocol.

Cell lysis of adherent growing cells in a culture dish:

Aspirate cell-culture medium completely, immediately add RNA 1 Lysis Buffer to the cell-culture dish. Take care to completely remove cell-culture medium in order to achieve optimal results with the lysis buffer.

To trypsinize adherent growing cells:

Aspirate cell-culture medium, add an equal amount of PBS to wash the cells. Aspirate PBS. Add 0.1 – 0.3% trypsin in PBS and incubate until cells have detached from the surface of the dish. Once cells have detached, add medium, transfer cells to an appropriate tube (not supplied), and pellet by centrifugation (5 min at 300 x *g*). Remove supernatant, add RNA 1 Lysis Buffer to the cell pellet.

#### Animal Tissue

Animal tissues are often solid and difficult to disrupt. They may need to be broken up mechanically and lysed using RNA 1 Lysis Buffer. Many tissue disruption techniques exist, a basic procedure is outlined below.

The Mortar and Pestle technique is a common way to disrupt frozen tissue. Grind the sample to a fine powder in the presence of liquid N<sub>2</sub>. Take care that the sample does not thaw during or after grinding. Add the frozen powder to an appropriate aliquot of RNA 1 Lysis Buffer containing β-mercaptoethanol and mix immediately. The broken-up tissue should then be homogenized using a SpinSmart Filter Column (purple ring) or by passing ≥ 5 through a 0.9 mm syringe needle.

Thawing of undisrupted animal tissue should only be allowed in the presence of RNA 1 Lysis Buffer during simultaneous mechanical disruption, e.g. with a rotor-stator homogenizer. The spinning rotor disrupts and simultaneously homogenizes the sample by mechanical shearing of DNA within seconds to minutes (homogenization time varies with sample type). The rotor tip should be completely submerged in order to avoid excess foaming. A 5 mm -7 mm diameter rotor can be used for homogenization in microcentrifuge tubes.

#### Bacteria and Yeast

Bacteria must be incubated in lysozyme solution and yeast should be disrupted in lyticase/zymolase solutions. The strong cell walls of these organisms must be digested or at least weakened for effective cell lysis by RNA 1 Lysis Buffer. For microorganisms with

extremely resistant cell walls, such as Gram-positive bacterial strains, additional treatment with lytic enzymes or optimization of the cultivation conditions may necessary. A SpinSmart Filter Column (purple ring) or a syringe-needle method should be used following lysis to homogenize the sample.

## 3.2. Elution Procedures

Modifications to Elution procedures

High yield

Perform two elution steps using the volume indicated in the individual protocol. 90-100% of bound nucleic acid will be eluted.

High yield and high concentration

Perform two elution steps with the initial eluate. Elute with the standard elution volume, then reapply this eluate over the column filter a second time.

RNases exist everywhere in our environment, including on general lab ware, fingerprints, and even in dust. A brief exposure to these enzymes can significantly degrade RNA. For short-term storage, eluted RNA should be frozen at  $-20^{\circ}\text{C}$ , for long-term storage freeze at  $-70^{\circ}\text{C}$ .

## 4. Storage Conditions and Preparation of Solutions

Buffers RNA 1, RNA 2, and RNA 3 contain guanidine thiocyanate. Wear gloves and goggles!

### rDNase

Store lyophilized RNase-free rDNase at +4°C on arrival (stable up to 1 year).

rDNase Working Solution:

Add indicated volume of RNase-free Water to the rDNase vial then incubate for 1 min at room temperature. Swirl the vials gently to completely dissolve the rDNase. **DO NOT** mix rDNase vigorously: rDNase is sensitive to mechanical forces. Dispense into aliquots and store at –18°C. The frozen working solution is stable for 6 months. Aliquots should not be frozen and thawed more than 3 times.

All other kit components should be stored at room temperature (20-25°C) and are stable up to one year. Storage at lower temperatures may cause precipitation of salts.

### Ethanol

70% and 96%-100% ethanol is not provided in the kit but must be provided by user.

### RNA 4 Wash Buffer

Add the indicated volume of 96 – 100% ethanol to RNA 4 Wash Buffer concentrate. Store at room temperature (20-25°C) for up to one year.

Catalg #	50 preps	250 preps
RNA 4 Wash Buffer (concentrate)	12.5 ml add 50 ml Ethanol (96% - 100% EtOH)	3 x 25 ml add 100 ml Ethanol (96% - 100% EtOH) to each bottle
rDNase (lyophilized)	1 vial  add 540 µl RNase-free Water	5 vials  add 540 µl RNase-free Water to each vial

### Reagents and equipment supplied by the user

- Ethanol (70% and 96-100%)
- β-mercaptoethanol
- Centrifuge for microcentrifuge tubes
- 1.5 ml microcentrifuge tubes
- Manual micropipettors and sterile, RNase-free tips
- Personal protection equipment (lab coat, gloves, goggles)
- Equipment for sample disruption and homogenization

## 5. Safety Instructions – Risk And Safety Phrases

The following components of SpinSmart RNA kits contain hazardous contents.

*Always wear gloves and goggles and follow the safety instructions given in this section.*

Component	Hazard Contents	Hazard Symbol	Risk Phrases	Safety Phrases
rDNase	rDNase, lyophilized	✘ <sup>*</sup> Xi*	May cause sensitization by inhalation and skin contact	R 42/43 S 22-24
RNA 1 Lysis Buffer	Guanidine thiocyanate	✘ <sup>*</sup> Xn*	Harmful by inhalation, in contact with skin and if	R 20/21/22 S 13
RNA 3 Wash Buffer	Guanidine thiocyanate	✘ <sup>*</sup> Xn*	Flammable. Harmful by inhalation, in contact with skin and if swallowed	R 10-20/21/22 S 7-13-16
RNA 2 Wash Buffer	Guanidine thiocyanate < 10 % + ethanol < 10%		Flammable	R 10 S 7-16

### Risk Phrases

R 10 Flammable

R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed

R 42/43 May cause sensitisation by inhalation and skin contact

### Safety Phrases

S 7 Keep container tightly closed

S 13 Keep away from food, drink and animal feedstuffs

S 16 Keep away from sources of ignition – No Smoking!

S 22 Do not breathe dust

S 24 Avoid contact with the skin

\* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

## 6. Spinsmart RNA Standard Protocol - From Cultured Cells And Tissue

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### 1 Homogenization of sample

Disrupt up to 30 mg of tissue (for sample amounts see section 3.1; for homogenization methods see section 3.1).

disrupt  
sample

Up to  $5 \times 10^6$  eukaryotic cultured cells are collected by centrifugation and lysed by directly adding Lysis Buffer RNA 1

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### 2 Cell lysis

Add 350  $\mu$ l RNA 1 Lysis Buffer and 3.5  $\mu$ l  $\beta$ -mercaptoethanol\* to the cell pellet or to ground tissue and vortex vigorously.

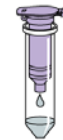
+ 350  $\mu$ l RNA 1  
Lysis Buffer  
+ 3.5  $\mu$ l  $\beta$ -me\*

*\* $\beta$ -mercaptoethanol is recommended for samples with high RNase levels.*

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### 3 Filtration of the lysate

**Filtration through SpinSmart Filter Columns (purple ring):** Place SpinSmart Filter Columns in a Collection Tube, apply the mixture, then centrifuge for 1 min at 11,000 x g.



In case of visible pellet formation, transfer supernatant (without any pellet solid) to a new 1.5 ml microcentrifuge tube.

1 min  
11,000 x g



**Important:** If higher amounts of cells ( $>1 \times 10^6$ ) or tissue ( $>10$  mg) must be processed, the lysate should first be homogenized using the 0.9 mm needle (20 gauge), followed by filtration through SpinSmart Filter Columns.

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4 Adjust RNA binding conditions

Discard the SpinSmart Filter Column (purple ring) and add 350  $\mu$ l ethanol (70%) to the homogenized lysate. Mix by pipetting up and down (5 times).

Alternatively transfer flow-through into a new 1.5 ml microcentrifuge tube (not provided), add 350  $\mu$ l ethanol (70%), and mix by vortexing (2 x 5 sec).



+ 350  $\mu$ l  
70% EtOH

mix

*After addition of ethanol a stringy precipitate may become visible which will not affect the RNA isolation. Mix the sample well and load all of the precipitate on the column as described in step 5. Do not centrifuge the ethanolic lysate before loading it onto the column!*

5 Bind RNA

For each preparation, take one SpinSmart RNA Binding Column (blue) placed in a Collection Tube. Pipet lysate up and down 2-3 times and load the lysate to the column. Centrifuge for 30 sec at 11,000  $\times$  g. Place the column in a new Collection Tube (2 ml).



load lysate

*Maximal loading capacity of the SpinSmart RNA Columns is 750  $\mu$ l. Repeat the procedure if larger volumes are to be processed.*



30 sec  
11,000  $\times$  g

6 Desalt silica membrane

Add 350  $\mu$ l RNA 2 Wash Buffer and centrifuge at 11,000  $\times$  g for 1 min to dry the membrane.



+ 350  $\mu$ l

RNA 2 Wash  
Buffer

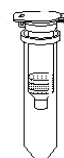
*If the column outlet has come into contact with the flow-through for any reason, discard the flow-through and centrifuge again for 30 sec at 11,000  $\times$  g.*



1 min  
11,000  $\times$  g

7 Digest DNA

Prepare DNase reaction mixture in a sterile 1.5 ml microcentrifuge tube (not provided): for each isolation, add 10  $\mu$ l reconstituted rDNase\* to 90  $\mu$ l Reaction Buffer for rDNase. Mix by flicking the tube.



+ 95  $\mu$ l DNase  
reaction  
mixture

Apply 95  $\mu$ l DNase reaction mixture directly onto the center of the silica membrane of the column. Incubate at room temperature for 15 min.

RT  
15 min

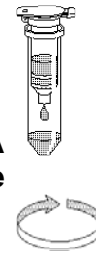
*\*See page 7 for rDNase preparation. 540  $\mu$ l RNase-free H<sub>2</sub>O must be added to 1 vial of lyophilized rDNase to reconstitute.*

**8** Wash and Dry silica membrane

**RNA 3 Wash**

**Add 200  $\mu$ l RNA 3 Wash Buffer to the SpinSmart RNA Binding Column. Centrifuge for 30 sec at 11,000  $\times$  g. Place the column into a new Collection Tube (2 ml).**

*RNA 3 Wash buffer will inactivate the rDNase.*



+ 200  $\mu$ l

RNA 3 Wash Buffer

30 sec  
11,000  $\times$  g

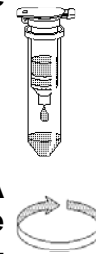
**RNA 4 Wash #1**

**Add 600  $\mu$ l RNA 4 Wash Buffer to the SpinSmart RNA Binding Column. Centrifuge for 30 sec at 11,000  $\times$  g. Discard flow-through and place the column back into the Collection Tube.**

**RNA 4 Wash #2**

**Add 250  $\mu$ l RNA 4 Wash Buffer to the SpinSmart RNA Column. Centrifuge for 2 min at 11,000  $\times$  g to dry the membrane completely. Place the column into a nuclease-free Collection Tube (1.5 ml, supplied).**

*If for any reason, the liquid level in the Collection Tube has reached the SpinSmart RNA Binding Column after centrifugation, discard flow-through and centrifuge again.*



+ 600  $\mu$ l RA3

30 sec  
11,000  $\times$  g

+ 250  $\mu$ l RA3

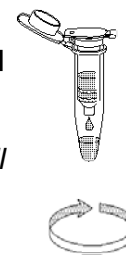
2 min  
11,000  $\times$  g

**9** RNA Elution

**Elute RNA in 60  $\mu$ l RNase-free Water (supplied) and centrifuge at 11,000  $\times$  g for 1 min.**

*Elution volumes between 40ul and 120ul may be used. Overall yield will decrease when using smaller volumes.*

*For further alternative elution procedures see Elution Procedure section on page 8.*



+ 60  $\mu$ l RNase-free Water

1 min  
11,000  $\times$  g

## 7. Spinsmart RNA Support Protocols

### 7.1. Total RNA Preparation From Biological Fluids (e.g. serum, culture medium)

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- 1 Homogenization of sample

**Not necessary**

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- 2 Cell lysis

**Add 350 µl RNA 1 Lysis Buffer to 100 µl of sample and vortex vigorously.**

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- 3 Filtration of the lysate

**Not necessary**

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- 4 Adjust RNA binding conditions

**Add 350 µl of ethanol (70%) to the lysate and mix by vortexing.**

**Proceed with step 5 of the SpinSmart RNA standard protocol (PAGE 9).**

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## 7.2. Total RNA Preparation From Up To 10<sup>9</sup> Bacterial Cells

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### 1 Homogenization of sample

**Resuspend the bacterial cell pellet (Gram-negative strains) in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8) containing 0.2 mg/ml lysozyme by vigorous vortexing. Incubate at 37°C for 10 min.**

*For preparation of RNA from Gram-positive bacteria, resuspend cells in 100 µl TE containing 2 mg/ml lysozyme. It may be necessary to optimize incubation time and lysozyme concentration, depending on the bacterial strain.*

*Note: It may be necessary to use a lower quantity of cells for the preparation as compared to standard protocol for cultured cells and animal tissue.*

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### 2 Cell lysis

**Add 350 µl Lysis Buffer RNA 1 and 3.5 µl β-mercaptoethanol\* to the suspension and vortex vigorously.**

*\*β-mercaptoethanol is recommended for samples with high RNase levels.*

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### 3 Filtration of lysate

**Place SpinSmart Filter Columns (purple ring) in Collection Tubes, apply mixture, and centrifuge for 1 min at 11,000 x g.**

**If a pellet forms, use flow-through of SpinSmart filter columns without disturbing the pellet of undissolvable cell debris.**

*Alternatively, the lysate may be passed ≥ 5 times through a 0.9 mm needle (20 gauge) fitted to a syringe.*

---

### 4 Adjust RNA binding conditions

**Add 350 µl of ethanol (70%) and proceed with step 5 of the SpinSmart RNA standard protocol (PAGE 9).**

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## 7.3. Total RNA Preparation From Yeast, Up To $5 \times 10^7$ Cells

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### 1 Homogenization of sample

**Harvest 2-5 ml of YPD culture ( $5,000 \times g$ ; 10 min). Resuspend pellet in sorbitol/lyticase buffer (50-100 U lyticase or zymolase in 1 M sorbitol/100 mM EDTA) and incubate at 30°C for 30 min. Pellet the resulting spheroplasts by centrifugation ( $1,000 \times g$ ; 10 min).**

*Note: It may be necessary to use a lower quantity of cells for the preparation. It may also be necessary to optimize incubation time and lyticase/zymolase concentration.*

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### 2 Cell lysis

**Add 350  $\mu$ l Lysis Buffer RNA 1 and 3.5  $\mu$ l  $\beta$ -mercaptoethanol\* to the suspension, vortex vigorously.**

*\* $\beta$ -mercaptoethanol is recommended for samples with high RNase levels.*

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### 3 Filtration of lysate

**Reduce viscosity and turbidity of the solution by filtration through the SpinSmart RNA Filter Columns. Place SpinSmart RNA Filter Columns in Collection Tubes, apply mixture, and centrifuge for 1 min at  $11,000 \times g$ .**

**If a pellet forms, use flow-through of SpinSmart RNA Filter without disturbing the pellet of undissolvable cell debris.**

*Alternatively, the lysate may be passed  $\geq 5$  times through a 0.9 mm needle (20 gauge) fitted to a syringe.*

**Proceed with step 4 of the SpinSmart RNA standard protocol (PAGE 10).**

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## 7.4. Total RNA Preparation From Paraffin Embedded Tissue

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- A** Place 10 mg of finely minced tissue into a 1.5 ml microcentrifuge tube (not provided).

Add 300  $\mu$ l Xylene, incubate 5 minutes with constant mixing at room temperature.

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- B** Centrifuge at maximum speed (13000 rpm) for 3 minutes to pellet the tissue. Discard the Xylene.
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- C** Repeat the steps A and B twice, for a total of three Xylene washes.
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- D** Add 300  $\mu$ l of 96% ethanol to the tube and incubate 5 minutes with constant mixing at room temperature.
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- E** Centrifuge at maximum speed (13,000 rpm) for 3 minutes to pellet the tissue. Discard the ethanol.
- 

- F** Repeat steps D and E, for a total of two ethanol washes.
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- G** Continue with step 1 of the SpinSmart RNA standard protocol (PAGE 9).
-

## 8. Troubleshooting

Problem	Suggestions
RNA is degraded/ no RNA obtained	<p><i>RNase contamination of your samples</i></p> <ul style="list-style-type: none"><li>• Create an RNase free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250°C before use.</li></ul>
Poor RNA quality or yield	<p><i>Reagents not used properly</i></p> <ul style="list-style-type: none"><li>• Reagents not properly reconstituted. Add the indicated volume of RNase-free Water to rDNase vial and 96%-100% ethanol to RNA 4 Wash Buffer Concentrate and mix. Reconstitute and store lyophilized rDNase according to instructions on Page 8.</li><li>• Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.</li><li>• No ethanol has been added after lysis. RNA Binding to silica membrane is only effective in the presence of ethanol.</li></ul> <p><i>Kit storage</i></p> <ul style="list-style-type: none"><li>• Reconstitute and store lyophilized rDNase according to instructions given on Page 8.</li><li>• Store all other kit components at room temperature. Storage at low temperatures may cause salt precipitation.</li><li>• Evaporation or contamination may occur if bottles are not stored with lids tightly closed.</li></ul>

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Problem	Suggestions
<p>Poor RNA quality or yield</p>	<p><i>Ionic strength and pH influence <math>A_{260}</math> absorption as well as ratio <math>A_{260/280}</math></i></p> <ul style="list-style-type: none"> <li>For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also:               <ul style="list-style-type: none"> <li>- Manchester, K L. 1995. Value of A260/A280 ratios for measurement of purity of nucleic acids. <i>Biotechniques</i> 19, 208-209.</li> <li>- Wilfinger, W W, Mackey, K and Chomczynski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. <i>Biotechniques</i> 22, 474-481.</li> </ul> </li> </ul> <p><i>Sample material</i></p> <ul style="list-style-type: none"> <li>Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid N<sub>2</sub>. Samples should always be kept at -70°C. Never allow tissues to thaw before addition of RNA 1 Lysis Buffer. Perform disruption of samples in liquid N<sub>2</sub>.</li> <li>Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use SpinSmart Filter Columns for homogenization of disrupted starting material.</li> </ul>
<p>Clogged SpinSmart column</p>	<p><i>Sample material</i></p> <ul style="list-style-type: none"> <li>Too much starting material used. Overloading may lead to decreased overall yield. Reduce amount of sample material or use larger volume of RNA 1 Lysis Buffer.</li> </ul>
<p>Poor RNA quality or yield</p>	<ul style="list-style-type: none"> <li>Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use SpinSmart Filter Columns (purple ring) for homogenization of disrupted starting material.</li> </ul>
<p>Genomic DNA contamination (continued)</p>	<p><i>rDNase not active</i></p> <ul style="list-style-type: none"> <li>Reconstitute and store lyophilized rDNase according to instructions given on Page 8.</li> </ul> <p><i>DNase solution not properly applied</i></p> <ul style="list-style-type: none"> <li>Pipet rDNase solution directly onto the center of the silica membrane.</li> </ul>
	<p><i>Too much cell material used</i></p> <ul style="list-style-type: none"> <li>Reduce quantity of cells or tissue used.</li> </ul>

<b>Problem</b>	<b>Suggestions</b>
Genomic DNA contamination (continued)	<p data-bbox="438 257 901 291"><i>DNA detection system too sensitive</i></p> <ul data-bbox="438 313 1380 448" style="list-style-type: none"><li>• The amount of DNA contamination is significantly reduced during the on-column digestion with rDNase. Purified RNA is not guaranteed to be 100% free of DNA. In very sensitive applications it might be possible to detect traces of DNA.</li></ul> <p data-bbox="486 481 1013 515">DNA detection with PCR increases with:</p> <ul data-bbox="486 548 1332 660" style="list-style-type: none"><li>- the number of DNA copies per preparation: single copy target - plastidial/ mitochondrial target &lt; plasmid transfected into cells</li><li>- decreasing PCR amplicon size</li></ul> <ul data-bbox="438 728 1364 907" style="list-style-type: none"><li>• Use larger PCR targets (e.g. &gt;500 bp) or intron spanning primers if possible.</li><li>• Use support protocol for subsequent rDNase digestion in solution (PAGE 8).</li></ul>
Poor performance in downstream experiments	<p data-bbox="438 952 798 985"><i>Carryover of ethanol or salt</i></p> <ul data-bbox="438 1008 1404 1243" style="list-style-type: none"><li>• Do not let the flow-through touch the column outlet after the second wash with RNA 4 Wash Buffer. Be sure to centrifuge at the corresponding speed for the respective time in order to completely remove ethanolic RNA 4 Wash Buffer.</li><li>• Make sure RNA 4 Wash Buffer is room temperature. Washing at lower temperatures lowers efficiency of salt removal.</li></ul> <p data-bbox="438 1288 805 1321"><i>Store isolated RNA properly</i></p> <ul data-bbox="438 1344 1388 1456" style="list-style-type: none"><li>• Eluted RNA should always be kept on ice for optimal stability. Freeze at -20°C for short term storage, Freeze at -70°C for long term storage.</li></ul>

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## 9. Ordering information

Cat. No.	Description	Qty.	Price
CM-610-50	SpinSmart™ Total RNA Mini Purification Kit Including on-column homogenizer filters and rDNase	50 preps	\$259.00
CM-610-250	SpinSmart™ Total RNA Mini Purification Kit Including on-column homogenizer filters and rDNase	250 preps	\$959.00
CM-600-50	RNA Miniprep <b>Bulk Columns with Collection Tubes only</b>	50 per pack	\$169.00
CM-600-250	RNA Miniprep <b>Bulk Columns with Collection Tubes only</b>	250 per pack	\$679.00