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SPEEDTOOLS TOTAL RNA EXTRACTION KIT

Designed for the rapid isolation of highly pure RNA from cells and tissue

Instructions for Use

(Cat. No. 21.211/2)

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

1. BASIC PRINCIPLE

The Kit is designed for the rapid isolation and purification of total RNA from cultured cells and tissue. The technology behind the Speedtools extraction kits is based on adsorption and desorption of nucleic acids to specially treated silica membrane in the presence of chaotropic salts.

The starting material i.e. cultured cells are lysed by incubation in a lytic solution containing large amounts of chaotropic ions. This lysis buffer immediately inactivates RNases, present in virtually all biological materials, and creates the appropriate conditions for binding of nucleic acids to the silica membrane of the RNA binding column. The obtained lysate is clarified by filtration through a filtering column. This step reduces the viscosity of the lysate which favour the process of binding to the RNA column. Contaminating DNA, which is also bound to the silica membrane, is removed by a digestion step in which a recombinant DNase is directly applied onto the column membrane. Simple washing steps with two different buffers remove salts, metabolites and macromolecular cellular components. Pure RNA is finally eluted under low ionic strength conditions with RNase-free H_2O.

The buffers formulated in the kit have been optimised to prevent degradation of the RNA during the isolation procedure. Moreover, all the steps can be performed at room temperature.

The eluate with the purified RNA should be treated with maximum care because RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints and dust. Keep RNA frozen at –20°C for short-term or at –70°C for long-term storage.

2. KIT CONTENTS

SPEEDTOOLS TOTAL RNA EXTRACTION KIT		
Catalogue Number	**21.211 (50 preps)**	**21.212 (250 preps)**
LYSIS BUFFER LR	25 ml	5X 25 ml
WASH BUFFER WR1	15 ml	5X 15 ml
WASH BUFFER WR2 (concentrated)	12.5 ml	5X 12.5 ml
DESALTING BUFFER DBR	25 ml	5X 25 ml
BUFFER FOR rDNase	7 ml	5X 7 ml
rDNase (free of RNases)	1 vial	5X 1 vial
RNase-free H_2O	15 ml	5X 15 ml
FILTERING COLUMNS (violet ring)	50	5X 50
RNA BINDING COLUMNS (blue ring) with collection tube	50	5X 50
COLLECTION TUBES (2 ml)	150	5X 150
COLLECTION TUBES (1.5 ml)	50	5X 50
PROTOCOL	1	1

3. KIT SPECIFICATIONS

Speedtools Total RNA Extraction Kit is recommended for the isolation of total RNA from **cultured cells** and **tissue**. Support protocols for the isolation of total RNA from **cell-free biological fluids**, **bacteria** and **yeasts** are included. The kit is also suitable for **clean-up of RNA** from reaction mixtures.

The Kit allows purification of RNA with an $A_{260/280}$ ratio generally exceeding 1.9 (measured in TE buffer pH 7.5).

Even biological samples e.g. mouse tissue (liver and brain), different tumor cell lines difficult to process will render high quality RNA.

The amount of DNA contamination is minimal due to on-column digestion with rDNase. However, in very sensitive applications it might be possible to detect traces of DNA.

Removal of DNA is checked by the following procedure: one million HeLa cells are subjected to RNA isolation according to the protocol. The obtained eluate is used as template for PCR detection of a 1 Kb fragment in a 30 cycle reaction. Generally no PCR fragment is obtained if the DNase is applied whereas a strong PCR fragment may be obtained if the DNase digestion is omitted. The probability of DNA detection with PCR increases with: the number of DNA copies per preparation (single copy target < plastidial/ mitochondrial target < plasmid transfected into cells) and decrease with the size of the PCR amplicon (big amplification size < small amplification size).

The standard protocol allows the purification of up to 70 µg of total RNA per RNA binding column from up to 5 x 10^6 cultured cells or 30 mg of tissue.

The isolated RNA can be used as template in a reverse transcription PCR (RT-PCR) reactions. We recommend using lower quantities of sample, 1-10% of the eluate of total RNA prepared from 1 x 10^6 cultured cells or 10 mg of tissue is sufficient as starting material. If possible, intron-spanning primers should be used for RT-PCR.

The isolated RNA is ready to use for applications like RT-PCR, Nothern, primer extension, arrays or RNase protection assays.

Table 1: General characteristics of the Kit

Sample size	Up to 5 x 10^6 cells 30 mg tissue
Average Yield	Up to 70 µg
Elution Volume	40 - 120 µl
Binding Capacity	100 µg
Time / Prep	< 30 min/6 preps
Spin Column Type	mini

The Kit can be used for preparing RNA from different amounts of sample material. For optimal results the volume of Lysis Buffer LR[1] (standard protocol step 1) and of ethanol (standard protocol step 4) should be adapted according to the following table. If 600 µl of Buffer LR and ethanol is used an additional loading step will be required, load the sample onto the column in two successive centrifugation steps.

Table 2: Recommended volumes of Lysis Buffer LR and Ethanol for different amounts of sample material.

SAMPLE	AMOUNT	LYSIS BUFFER LR	ETHANOL
Cultured animal or human cells (e.g. HeLa cells)	up to 5 x 10^6	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*later*®	up to 20 mg 20 - 30 mg	350 µl 600 µl	350 µl 600 µl
Samples hard to lyse	up to 30 mg	600 µl	600 µl

[1] The volume of Lysis Buffer LR included in the Kit is not sufficient to perform all preparations with 600 µl. If required, additional lysis buffer LR can be ordered separately.

Depending on sample type, the average yield is around 5 µg -70 µg total RNA (see Table 3). The $A_{260/280}$ ratio, indicates purity of the RNA, generally this value exceeds 1.9.

Table 3: Overview on average yields of total RNA isolation using the Kit

Sample	Average yield (µg)
8 x 10^4 HeLa cells	1.5
4 x 10^5 HeLa cells	4
1 x 10^6 HeLa cells	14
2 x 10^6 HeLa cells	21
2.5 x 10^6 HeLa cells	25
5 x 10^6 HeLa cells	50

4. HANDLING, PREPARATION, AND STORAGE OF STARTING MATERIALS

RNA is not protected against degradation until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Therefore it is important that samples are flash frozen in liquid N_2 immediately and stored at –70°C, or processed as soon as possible.

Samples can be stored in Lysis Buffer LR after disruption at –70°C for up to one year, at 4°C for up to 24 hours or up to several hours at room temperature. Frozen samples are stable up to 6 months. Frozen samples in Buffer LR should be thawed slowly before starting with the isolation of total RNA.

NOTE

Wear gloves at all times during the preparation and change them frequently.

CULTURED ANIMAL CELLS

Collect the cells by centrifugation and directly lyse them by adding Buffer LR according to Step 2 of the Standard Protocol.

Cell lysis of adherent growing cells in a culture dish

To allow full activity of the lysis buffer the cell-culture medium has to be removed completely. Aspirate the cell-culture medium, and continue immediately with the addition of Lysis Buffer LR to the cell-culture dish.

To trypsinize adherent growing cells

Aspirate the cell-culture medium, and wash the cells adding an equal amount of PBS. Aspirate PBS and add 0.1 – 0.3% trypsin in PBS. Incubate for an appropriate time to detach the cells from the dish surface. After cell detachment, add medium, transfer cells to an appropriate tube (not supplied), and pellet the cells by centrifugation for 5 min at 300 x *g*. Remove supernatant and continue with the addition of lysis buffer to the cell pellet.

ANIMAL TISSUES

If the animal tissue is solid it must be broken up mechanically. Depending on the disruption method, the viscosity of the lysed sample has to be reduced further for obtaining optimal results. For an efficient RNA preparation it is essential that all the RNA contained in the sample must be released from the cells by disruption and that the viscosity of the sample be reduced by homogenization.

The most commonly used technique for disruption of animal tissues is grinding with a pestle and mortar. Grind the sample to a fine powder in the presence of liquid N_2. Take care that the sample does not thaw during the grinding process or after grinding during weighing. Add the frozen powder to an appropriate aliquot of Buffer LR containing β-mercaptoethanol and mix immediately. The broken-up tissue must then be homogenized with a Filtering Column or by passing ≥ 5 times through a 0.9 mm syringe needle.

Thawing of undisrupted animal tissue should be exclusively done in the presence of Buffer LR during simultaneous mechanical disruption. This ensures that the RNA is not degraded by RNases before the

preparation has started. When using any kind of commercial homogenizer keep the rotor tip well submerged in the mixture in order to avoid excess foaming formation.

BACTERIA AND YEASTS

Bacteria and yeast have to be incubated in lysozyme or lyticase/zymolase solutions, respectively (see the support protocol in sections 8C and 8D). By this treatment, the robust cell walls of these organisms are digested or at least weakened, which is essential for effective cell lysis by Buffer LR. For microorganisms with extremely resistant cell walls – like some Gram-positive bacterial strains – it may be necessary to optimize the conditions of the treatment with the lytic enzymes or the cultivation conditions. After lysis, homogenization is achieved by the use of the Filtering Columns or the syringe-needle method.

5. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Ethanol 70%
- Ethanol 96-100%
- β-mercaptoethanol
- Centrifuge for microcentrifuge tubes
- Manual pipettes and sterile RNase-free tips
- 1.5 ml microcentrifuge tubes
- Equipment for sample disruption and homogenization

6. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

NOTE

Buffers LR, WR1, WR2 and DBR **contain guanidine thiocyanate therefore wear gloves and goggles**.

On arrival store lyophilized **rDNase** (free of RNases) at **4°C**. rDNase is stable up to 1 year at 4°C.

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.

Before starting any protocol with **SPEEDTOOLS TOTAL RNA EXTRACTION KIT** prepare the following reagents:

- **rDNase** (free of RNases)**:** Add 540 µl of RNase-free H_2O to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the DNase. Be careful not to mix rDNase vigorously as **rDNase is sensitive to mechanical agitation**. Dispense into aliquots and store at –18°C. The frozen working solution is stable for 6 months. Do not freeze/thaw the aliquots more than three times.
- **Buffer WR2:** Add 50 ml of 96–100% ethanol to Buffer WR2 Concentrate. Store the obtained Buffer WR2 at room temperature (20-25°C) for up to one year.

7. OTHER ELUTION PROTOCOLS

It is possible to adapt the elution method and volume of water used for the subsequent application of interest. In addition to the elution method described in each individual protocol (recovery rate about 70-90%) several modifications are possible.

High yield: Perform two elution steps with the volume indicated in the individual protocol. About 90-100% of bound nucleic acid will be eluted.

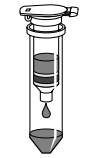
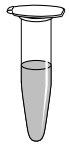
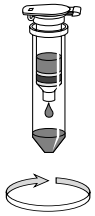
High yield and high concentration: Elute with the standard elution volume and apply the eluate once more onto the column for re-elution.

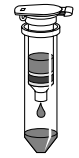
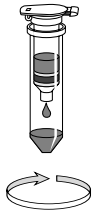
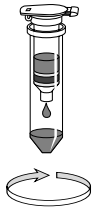
Eluted RNA should immediately be kept on ice for optimal stability to avoid degradation for the omnipresent RNases present in lab ware, fingerprints, dust, etc. For short-term storage freeze the purified RNA at –20°C, for long-term storage freeze at –70°C.

8. INSTRUCTION FOR USE

A. STANDARD PROTOCOL Total RNA purification from cultured cells and tissue

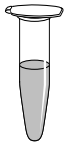
Before starting the protocol prepare rDNase solution and Buffer WR2 (see section 6).

STEP	DESCRIPTION		
1	**SAMPLE PREPARATION** *Tissue* Disrupt up to 30 mg of tissue (see section 4). *Cultured cells* Collect up to 5 x 10^6 eukaryotic cultured cells by centrifugation.		DISRUPT SAMPLE
2	**CELL LYSIS** Add **350 µl Buffer LR** and **3.5 µl β-mercaptoethanol** to the cell pellet or to ground tissue and vortex vigorously.		350 µl BUFFER LR + 3.5 µl β -mercapto ethanol
3	**FILTRATION OF THE LYSATE** Place a **filtering column** (violet ring) in a collection tube **apply the lyslate** and centrifuge for **1 min at 11,000 x *g***. In this way the lysate will be clarify and its viscosity will be reduced. *The lysate may be passed alternatively ≥ 5 times through a 0.9 mm needle (20 gauche) fitted to a syringe.* In case of visible pellet formation (depending on sample amount and nature) transfer supernatant without any formed pellet to a new 1.5 ml microcentrifuge tube (not supplied) **NOTE: To process higher amounts of cells (> 1 x 10^6) or tissue (>10 mg), before filtering the lysate should be homogenized using the 0.9 mm needle (20 gauche).**	 	Load lysate into a filtering column (violet ring) *1 min, 11,000 × g*
4	**ADJUST RNA BINDING CONDITIONS** Discard the filtering column and add **350 µl ethanol 70%** to the clarified lysate and **mix** by pipetting up and down (5 times). *Alternative transfer flow-through into a new 1.5 ml microcentrifuge tube (not provided), add 350 µl ethanol 70%, and mix by vortexing (2 x 5 sec).* After addition of ethanol a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to disaggregate any precipitate by mixing. Do not centrifuge the ethanolic lysate to avoid pelleting the precipitate.		+ 350 µl ETHANOL 70% *Mix*
5	**BIND RNA** For each preparation, take one **RNA binding column** (blue ring) placed into collection tube. Pipet the **lysate mixture** up and down 2-3 times and **load** it into the column. Centrifuge for **30 sec at 11,000 × *g***. Discard collection tube with flow-through and place the column back into a new collection tube. **Repeat the procedure if large volumes are to be processed**		Load mixture into a RNA binding column (blue ring) *30 sec, 11,000× g*
6	**DESALT SILICA MEMBRANE** Add **350 µl Buffer DBR** and centrifuge at **11,000 x *g* for 1 min** to dry the membrane**.** Salt removal will make the following step rDNase digestion more efficient. If for any reason the column outlet has come into contact with the flow-through, discard the flow-through and centrifuge again for 30 sec at 11,000 x *g*.		+ 350 µl BUFFER DBR *1 min, 11,000 × g*

7	**ON-COLUMN DNA DIGESTION** Prepare DNase Reaction Mixture: in a sterile 1.5 ml microcentrifuge tube add **10 µl reconstituted rDNase** to **90 µl Buffer for rDNase. Mix** by flicking the tube. Apply **95 µl DNase Reaction Mixture** directly onto the center of the silica membrane of the column. **Incubate at room temperature for 15 min.**		+ 95 µl DNase Reaction Mixture RT 15 min
8	**WASH AND DRY SILICA MEMBRANE** **1st Wash** Add **200 µl Buffer WR1.** Centrifuge **30 secs at 11,000 x *g*.** Place the column into a new collection tube (2 ml). *Buffer WR1 inactivate the rDNase.* **2nd Wash** Add **600 µl Buffer WR2.** Centrifuge **30 secs at 11,000 x *g*. Discard flow through** and place the column back into the collection tube. **3rd Wash** Add **250 µl Buffer WR2.** Centrifuge **2 min at 11,000 x *g*** to dry the membrane completely. **Discard flow through** and place the column back into a nuclease free collection tube (1.5 ml).		+ 200 µl BUFFER WR1 *30 sec,11,000 xg* + 600 µl BUFFER WR2 *30 sec,11,000 × g* + 250 µl BUFFER WR2 *2 min,11,000 × g*
9	**ELUTE HIGHLY PURE RNA** Dispense **60 µl RNase-free H_2O** directly onto the silica membrane. Centrifuge **1 min at 11,000 x *g*.** The eluate contains your pure RNA sample. If higher RNA concentrations are desired, elution can be done with 40 µl RNase-free H_2O. Overall yield, however, will decrease when using smaller volumes. **For further alternative elution procedures see Section 7**		+ 60 µl RNase-free H_2O *1 min, 11,000 × g*

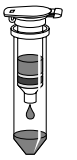
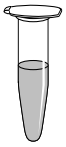
B. SUPPORT PROTOCOL: Total RNA preparation from biological fluids (e.g. serum, culture medium)

Before starting the protocol prepare rDNase solution and Buffer WR2 (see section 6).

STEP	DESCRIPTION		
1	**SAMPLE LYSIS** Sample homogenization is not necessary. Add **350 µl Buffer LR** to **100 µl of sample** and **vortex vigorously.**		100 µl Starting material + 350 µl BUFFER LR *vortex*
2	**ADJUST RNA BINDING CONDITIONS** Add **350 µl ethanol 70%** to the lysate and **mix** by vortexing.		+ 350 µl ETHANOL 70% *vortex*
	Proceed with Step 5 of the *Standard Protocol*.		

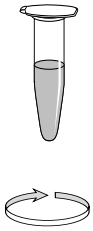
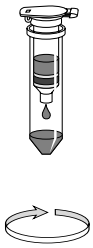
C. SUPPORT PROTOCOL Total RNA preparation from up to 10^9 bacterial cells

Before starting the protocol set a water bath or incubator at 37ºC. Prepare rDNase solution and Buffer WR2 (see section 6).

STEP	DESCRIPTION		
1	**SAMPLE PREPARATION** • For **Gram negative** strains resuspend the bacterial cell pellet 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 **37ºC** for **10 min.** • For preparation of RNA from **Gram-positive** bacteria, resuspend cells in **100 µl TE buffer** containing **2 mg/ml lysozyme**. It may be necessary to **optimize incubation** time and lysozyme concentration, depending on the bacterial strain. NOTE: Because of the higher concentration of genome equivalents in a nucleic acid preparation of bacteria compared with eukaryotic material, it may be necessary to use a lower quantity of cells for the preparation.		CELL PELLET + 100 µl BUFFER TE (with LYSOZYME) 37ºC, 10 min
2	**CELL LYSIS** Add **350 µl Buffer LR** and **3.5 µl β-mercaptoethanol** to the cell suspension and **vortex** vigorously.		350 µl BUFFER LR + 3.5 µl β -mercapto ethanol
3	**FILTRATION OF THE LYSATE** Place a **filtering column** (violet ring) in a collection tube **apply the lysate** and centrifuge for **1 min at 11,000 x *g***. In this way the lysate will be clarify and its viscosity will be reduced. *In case of visible pellet formation recover and use flow-through of the filtering column without disturbing the pellet of undissolved cell debris.* Alternatively, the lysate may be passed ≥ 5 times through a 0.9 mm needle (20 gauge) fitted to a syringe.	 	Load lysate into a filtering column (violet ring) *1 min, 11,000 × g*
4	**ADJUST RNA BINDING CONDITIONS** Discard the filtering column and add **350 µl ethanol 70%** to the clarified lysate and **mix** by pipetting up and down (5 times).		+ 350 µl ETHANOL 70% *Mix*
	Proceed with Step 5 of the *Standard Protocol*.		

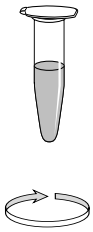
D. SUPPORT PROTOCOL Total RNA preparation from up to 5 x 10^7 yeast cells

Before starting the protocol set a water bath or incubator at 30ºC. Prepare rDNase solution and Buffer WR2 (see section 6).

STEP	DESCRIPTION		
1	**SAMPLE PREPARATION** **Harvest 2-5 ml of YPD culture** (5,000 x *g*; 10 min). Resuspend the pellet in **sorbitol/lyticase buffer** (50-100 U lyticase or zymolase in 1 M sorbitol/100 mM EDTA) and incubate at **30ºC** for **30 min**. Pellet the resulting spheroplast by centrifugation (1,000 x *g*; 10 min). *It may be necessary to optimize incubation time and lyticase/zymolase concentration depending on the yeast strain.* NOTE: Due to the much higher concentration of genome equivalents in a nucleic acid preparation of yeasts compared with cultured cells or tissue material, it may be necessary to use a lower quantity of cells for the preparation.		CELL PELLET + SOBITOL/ LYTICASE BUFFER 30ºC, 30 min *10 min, 1,000 ×g*
2	**CELL LYSIS** Add **350 µl Buffer LR** and **3.5 µl β-mercaptoethanol** to the cell suspension and **vortex** vigorously.		350 µl BUFFER LR + 3.5 µl β -mercapto ethanol
3	**FILTRATION OF THE LYSATE** Place a **filtering column** (violet ring) in a collection tube **apply the lysate** and centrifuge for **1 min at 11,000 x *g***. In this way the lysate will be clarify and its viscosity will be reduced. *In case of visible pellet formation recover and use flow-through of the filtering column without disturbing the pellet of undissolved cell debris.* Alternatively, the lysate may be passed ≥ 5 times through a 0.9 mm needle (20 gauge) fitted to a syringe.		Load lysate into a filtering column (violet ring) *1 min, 11,000 × g*
	Proceed with Step 4 of the *Standard Protocol*.		

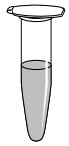
E. SUPPORT PROTOCOL Total RNA preparation from paraffin embedded tissue

Before starting the protocol prepare rDNase solution and Buffer WR2 (see section 6).

STEP	DESCRIPTION		
1	**REMOVAL OF PARAFFIN** **A)** Take 10 mg of disrupted tissue into a 1.5 ml centrifuge tube (not provided). Add **300 µl Xylene** and incubate **5 min** with **constant mixing** at room temperature. Centrifuge at **max speed** for **3 min** to pellet the tissue. **Discard the Xylene**. **Repeat twice**, for a total of three Xylene washes. **B)** Add **300 µl** of **ethanol 96%** to the tube and incubate **5 min** with constant **mixing** at room temperature. Centrifuge at max speed for 3 min to pellet the tissue. **Discard the ethanol**. **Repeat once**, for a total of two ethanol washes		10 mg disrupted tissue + 3 Times Xylene (300 µl) Wash *3 min, v max* 2 Times Ethanol 96% (300 µl) Wash *3 min, v max*
	Proceed with Step 1 of the *Standard Protocol*.		

F. SUPPORT PROTOCOL Clean-up of RNA from reaction mixtures

Before starting the protocol prepare rDNase solution and Buffer WR2 (see section 6).

STEP	DESCRIPTION		
1	**SAMPLE PREPARATION** A) For sample volumes smaller than 100 µl fill up with RNase-free H_2O until reaching 100 µl. B) If different samples with varying volumes between 100-200 µl are purified, RNA samples should be filled up with RNase-free H_2O to a uniform volume (e.g. 200 µl).		Prepare Sample
2	**PREPARATION OF LYSIS-BINDING PREMIX BUFFER** The ratio of the Lysis-Binding Premix buffer (Lysis buffer LR to Ethanol 96-100%) is 1 vol:1 vol. For each **100 µl RNA sample mix 300 µl of Lysis Buffer LR and 300 µl ethanol 96-100%.** If 200 µl of RNA sample is processed mix 600 µl of Lysis Buffer LR and 600 µl ethanol 96-100%. *If multiple samples are processed, the preparation of a master with the premix buffer is recommended.*		Prepare Lysis-Binding Premix Buffer
3	**ADJUST RNA BINDING CONDITIONS** For a **100 µl of RNA sample** add **600 µl of Lysis-Binding Premix Buffer**. Consequently if **200 µl of RNA sample** are processed add **1200 µl of Lysis-Binding Premix Buffer.** *Maximum loading capacity of RNA Binding Columns is 750 µl. Repeat the loading step if larger volumes are to be processed.* After addition of ethanol or Premix Buffer a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to mix thoroughly and apply sample as homogenous solution onto the column.		Reaction Mixture + Lysis-Binding Premix Buffer
	Proceed with Steps 5, 8, and 9 of the Standard Protocol. In this particular case the steps 6 and 7 of the respective protocol may be omitted.		

G. SUPPORT PROTOCOL Total RNA preparation from RNA*later*® treated samples

Before starting the protocol prepare rDNase solution and Buffer WR2 (see section 6).

STEP	DESCRIPTION		
1	**SAMPLE PREPARATION** Remove RNA*later*® solution. Cut an appropriate amount of tissue.		Prepare Sample
2	**CELL LYSIS** Add **350 µl Buffer LR** and **3.5 µl β-mercaptoethanol** to the sample. Disrupt the sample material by using an appropriate homogenizer.		350 µl BUFFER LR + 3.5 µl β -mercapto ethanol
	Proceed with Step 3 of the *Standard Protocol.*		

H. SUPPORT PROTOCOL rDNase digestion in solution

The on-column rDNase digestion in the standard protocol is already very efficient resulting in a minimal residual DNA content of the purified RNA. This DNA will not be detectable in most downstream applications. Despite this, there are still certain applications which require even lower contents of residual DNA. However, removal of DNA to a completely undetectable level is challenging and the efficiency of an on-column DNA digestion is sometimes not sufficient for downstream applications requiring lowest residual content of DNA.

A typical example for such a demanding application is an RT-PCR reaction in which the selected primer does not differentiate between cDNA (derived from RNA) and contaminating genomic DNA. Especially if,

- High copy number targets are analysed (e.g. multi gene family, mitochondrial, plastidal or plasmid targets from transfections)
- The target gene is of a very low expression level
- The amplicon is relatively small (<200 bp)

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent repurification of the RNA (in order to remove buffer, salts, DNase and digested DNA) are usually required.

The high quality, recombinant rDNase (free of RNases) included in the Kit facilitates such a digestion in solution in order to remove even traces of contaminating DNA.

STEP	DESCRIPTION
1	**DIGEST DNA (Reaction Set-up)** Add 6 µl Buffer for rDNase and 0.6 µl reconstituted rDNase to 60 µl eluted RNA.
2	**INCUBATION** Incubate at 37ºC for 10 min.
3	**REPURIFICATION OF RNA** Repurify RNA with a suitable RNA clean-up procedure *(Support Protocol 8F)* or by ethanol precipitation. *Ethanol precipitation procedure: Add 0.1 volume of 3 M sodium acetate, pH 5.2 and 2.5 volumes of 96-100% ethanol to one volume of sample. Mix thoroughly and incubate several minutes (for high RNA concentration) to several hours (for low RNA concentration) at -20ºC or 4ºC. Centrifuge for 10 min at max. speed. Wash RNA pellet with 70% ethanol. Dry RNA pellet and resuspend RNA in RNase-free H_2O.*

9. SIMULTANEOUS ISOLATION OF RNA AND DNA (RNA/DNA BUFFER TOOL SET)

The RNA/DNA Buffer Tool Set is a support set of buffers (Cat. No. 21.213 not included in the kit) for the isolation of RNA and DNA in conjunction with the Speedtools Total RNA Extraction Kit.

The technology behind this buffer set enables successive elution of DNA and RNA from one RNA Binding Column. After wash steps DNA and RNA eluted sequentially. DNA is eluted with a low salt buffer, which selectively elutes DNA and keeps RNA on the column. Eluted DNA is immediately ready for downstream applications without further purification. After DNA elution, residual on-column DNA is digested on the RNA Binding Column as described in the standard protocol. The column is washed and pure RNA is eluted in RNase-free H_20.

10. TROUBLESHOOTING

Problem	Possible cause and suggestions
RNA is degraded/no RNA obtained	***RNase contamination*** • 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 250ºC before use.
Poor RNA quality or yield	***Reagents not applied or restored properly*** • Reagents not properly restored. Add the indicated volume of RNase-free H_2O to rDNase vial, and 96% ethanol to Buffer WR2 (concentrated). Mix reagents following the instructions in section 6. Reconstitute and store lyophilized rDNase according to the instructions given in section 6. • Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added. • No ethanol has been added after lysis. Binding of RNA to the silica membrane is only effective in the presence of ethanol. ***Kit storage*** • Reconstitute and store lyophilized rDNase according to instructions given in section 6. • Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination. ***Ionic strength and pH influence A_{260} absorption as well as ratio $A_{280/260}$*** • For adsorption measurement, use 5 mM Tris, pH 8.5 as diluent. ***Sample material*** • Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid N_2. Samples should always be kept at -70ºC. Never allow tissues to thaw before addition of Buffer LR. Perform disruption of samples in liquid N_2. • Insufficient disruption and/or homogenization of starting material. Ensure thoroughly sample disruption and use the filtering columns for easy homogenization of disrupted starting material
Clogged column/ Poor RNA quality or yield	***Sample material*** • Too much starting material. Overloading may lead to decreased overall yield. Reduce amount of sample material or use larger volume of Lysis Buffer LR. • Insufficient disruption and/or homogenization of starting material. Ensure thoroughly sample disruption and use the filtering columns for easy homogenization of disrupted starting material.

Problem	Possible cause and suggestions
Contamination of RNA with genomic DNA	***rDNase not active*** • Reconstitute and store lyophilized rDNase according to instructions given in Section 6. ***DNase solution not properly applied*** • Pipet rDNase solution directly onto the center of the silica membrane. ***Too much cell material used*** • Reduce quantity of cells or tissue used. ***DNA detection system too sensitive*** • The amount of DNA contamination is significantly reduced during the on-column digestion with rDNase. Anyhow it cannot be guaranteed that the purified RNA is 100% free of DNA, therefore in very sensitive applications it might be possible to detect DNA. • Use larger PCR targets (e.g. >500 bp) or intron spanning primers if possible. • For subsequent rDNase digestion in solution use support protocol H
Suboptimal performance of RNA in downstream experiments	***Carryover of ethanol or salt*** • Do not let the flow-through touch the column outlet after the second buffer WR2 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer WR2 completely. • Check if Buffer WR2 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer WR2. ***Store isolated RNA properly*** • Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases will degrade the isolated RNA. For short term storage freeze at -20ºC, for long term storage freeze at -70ºC.

11. SAFETY INSTRUCTIONS

The following components of the SPEEDTOOLS TOTAL RNA EXTRACTION KIT contain hazardous contents. Wear gloves and goggles and follow the safety instructions given in this section.

Reagent	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
rDNase	rDNase lyophilized	 Xi*	May cause sensitization by inhalation and skin contact Do not breathe dust Avoid contact with the skin	R 42/43	S 22-S 24
Buffer WR1	guanidine thiocyanate	 Xn*	Harmful by inhalation, in contact with the skin and if swallowed Keep away from food, drink and animal feedstuffs	R 20/21/22	S 13
Buffer WR2	guanidine thiocyanate	 Xn*	Flammable Harmful by inhalation, in contact with the skin and if swallowed Keep container tightly closed Keep away from food, drink and animal feedstuffs Keep away from sources of ignition-No Smoking!	R 10-R 20/21/22	S 7-S 13-S 16
Desalting Buffer DBR	<10% guanidine thiocyanate <10% ethanol		Flammable	R10	S 7-S 16

12. ORDERING INFORMATION

SPEEDTOOLS TOTAL RNA EXTRACTION KIT	Cat. No. 21.211	50 preps
SPEEDTOOLS TOTAL RNA EXTRACTION KIT	Cat. No. 21.212	250 preps
RNA/DNA BUFFER TOOL SET	Cat. No. 21.213	100 preps

13. PRODUCT USE RESTRICTION AND WARRANTY

1. Product for research purposes and *in vitro uses* only.
2. No claim or representation is intended for its use to identify any specific microorganism or for clinical use (diagnostic, prognosis, therapeutic, or blood bank).
3. It is rather the responsibility of the user to verify the use of the kit for a specific application range as the performance characteristic of this kit has not been verified for a specific microorganism.
4. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure RNA.

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

5. BIOTOOLS warrants to meet the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
6. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.
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