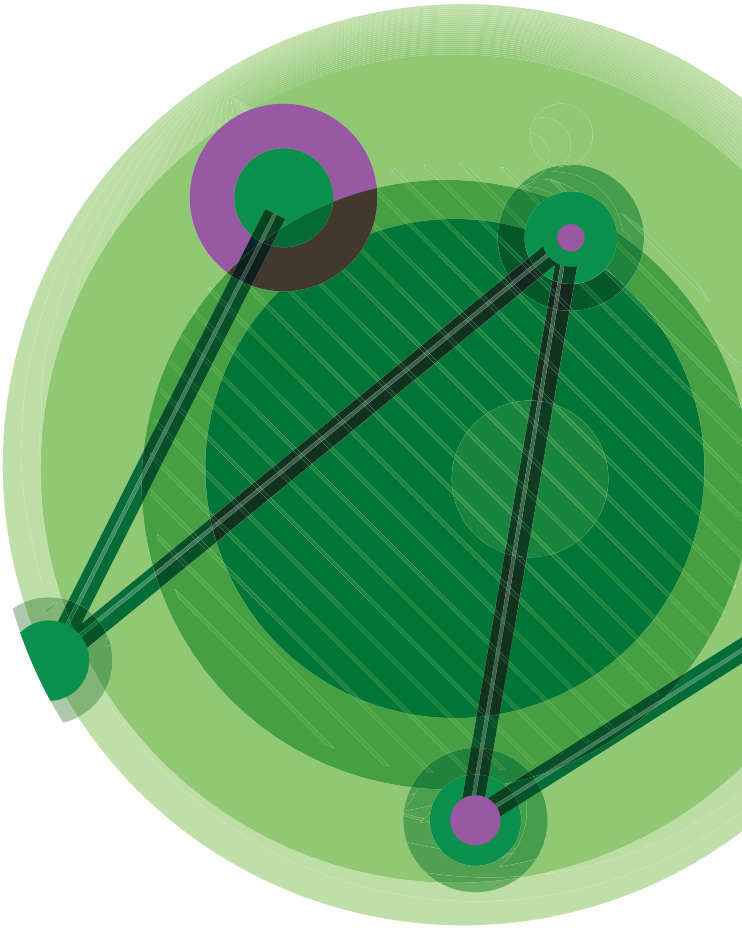
EXTRACTME®
TOTAL RNA MICRO SPIN KIT

Cat. No. EM31
Version: 1.2016

Kit for total RNA isolation from animal tissue and cell culture in low elution volume



I. INTENDED USE

The ***EXTRACTME*** **TOTAL RNA MICRO SPIN KIT** is designed for the rapid and efficient purification of high quality RNA up to 15 mg of tissue (fresh or frozen) and up to 10^6 cultured cells with an extremely low elution volume of only 5 µl. The isolation protocol and buffer formulations were optimized for high isolation efficiency and purity of RNA. The product is intended for research use only.

II. COMPONENTS OF THE KIT AND STORAGE CONDITIONS

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS	3 ISOLATIONS (DEMO)
Catalogue number	EM31-010	EM31-050	EM31-250	EM31-D
⚠ **RLys Buffer**[*] (RNA Tissue Lysis Buffer)	3.5 ml	18 ml	88 ml	1.1 ml
⚠ **DNase I, RNase-free** (lyophilized)	1 tube	2 tubes	10 tubes	–
⚠ **Nuclease-free water**	55 µl	260 µl	1.3 ml	–
⚠ **10x DNase I Reaction Buffer**	450 µl	2.3 ml	11.3 ml	–
RW Buffer (RNA Wash Buffer)	13.5 ml	68 ml	338 ml	3 ml
⚠ **REB** (RNA Elution Buffer)	2 ml	10 ml	5x 10 ml	600 µl
RNA Homogenizing Columns H	10 pcs	50 pcs	5x 50 pcs	3 pcs
RNA Purification Micro Spin Columns	10 pcs	50 pcs	5x 50 pcs	3 pcs
Collection Tubes (2 ml)	10 pcs	50 pcs	5x 50 pcs	3 pcs

[*] Before starting the isolation procedure **100% ß-mercaptoethanol** may be added to the **RLys Buffer**, to a final concentration of 1%. The combined RLys Buffer and ß-mercaptoethanol will remain stable at 2-8°C for four weeks. Therefore, when isolating in parts, transfer appropriate volume of the **RLys Buffer** for one isolation procedure to a separate RNase-free bottle/tube and add ß-mercaptoethanol. Marking the bottle after adding ß-mercaptoethanol is recommended.

⚠ DNase I is shipped lyophilized. **The DNase I lyophilized should be stored at +4°C**. Before using for the first time, reconstitute each vial of the DNase I lyophilizate in 130 µl of Nuclease-free water (EM31-050, EM31-250) or in 55 µl of Nuclease-free water (EM31-010). Incubate 1 minute at room temperature. Mix carefully by inverting the tube several times. DNase I is sensitive to physical denaturation. Therefore, **do not vortex** DNase I solution. Divide it into aliquots to avoid excessive freeze-thawing. Do not freeze/thaw more than three times. **After reconstitution** the DNase I should be kept **at -20°C** and it is stable for 6 months.

⚠ **RLys, Nuclease-free water, 10x DNase I Reaction Buffer, REB Buffer should be stored at +4°C. Protect the RLys from the sunlight!**

Both Homogenizing and Purification Columns can be stored either at +4°C or at room temperature.
In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing. Under proper storage conditions, the kit will remain stable for at least 12 months.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- 96-100% ethanol PFA
- 1.5-2ml RNase-free microcentrifuge tubes
- automatic pipettes and pipette tips (RNase-free)
- disposable gloves
- microcentrifuge with rotor for 1.5-2 ml (≥11k x g)
- vortex mixer
- freezing racks (< 7°C) for 1.5-2 ml tubes or dish enabling incubation at cooling conditions.

May be necessary:

- scissors, scalpel
- bead-beating tubes with ceramic filling (cat. no. HPLM100)
- tissue homogenizer for 2 ml tubes
- mechanical homogenizer with knives
- thermomixer, shaking orbit of 2 mm minimum
- 50-75 ml smooth-stroke mortar with fitted piston
- liquid nitrogen or dry ice
- vortex mixer with a 2 ml tube adaptor
- centrifuge with a rotor for 10-15 ml tubes (physiological fluids, cell cultures)
- 3% hydrogen peroxide or < 0.5% sodium hypochlorite
- 100% β-mercaptoethanol

IV. PRINCIPLE

The ***EXTRACTME*** **TOTAL RNA MICRO SPIN KIT** utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids at high concentration of chaotropic salts. In the first isolation step, the tissue is homogenized in order to disintegrate intercellular bonds (epithelial tissue) and fragmentize high-molecular proteins (muscle or connective tissue). Then the homogenate is lysed with guanidine thiocyanate and detergents. Any RNases are inactivated by guanidine thiocyanate. The homogenate is separated from the undigested tissue/cell remains by centrifugation and on the Homogenizing Column. The RNA is bound to the Purification Micro Spin Column membrane by addition of ethanol. The two-step washing stage effectively removes impurities and enzyme inhibitors. The purified RNA is eluted using a low ionic strength buffer or RNase-free water (pH 7.0-9.0) and can be used directly in all downstream applications such as RT-PCR, Northern blotting, RT-qPCR and so forth.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACTME*** **TOTAL RNA MICRO SPIN KIT** is tested using standard QC procedures. The purified RNA concentration, quality and stability are evaluated by gel electrophoresis and spectrophotometer. In addition, the functional quality is tested by reverse transcription and qPCR.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL

- → fresh or frozen tissue (stored at -80°C): up to 15 mg
- → tissue preserved in RNase inactivating buffers: up to 15 mg
- → cell culture: up to 10^6 cells

BINDING CAPACITY

Approx. 30 µg RNA

TIME REQUIRED

- → 16-20 minutes (lysis and homogenization time not included)
- → 30-60 minutes for homogenization in liquid nitrogen
- → 30-40 minutes for mechanical homogenization (ceramic beads)
- → 15 minutes for optional DNase I treatment

DNA PURITY

A_{260}/A_{280} ratio = 1.9 – 2.1

VII. SAFETY PRECAUTIONS

- Tissue is treated as a biohazardous material on account of its potential pathogen content or health and life-threatening substances. While working with tissue and cell cultures, compliance with all the safety requirements for working with biohazardous material is essential.
- Conducting the entire isolation procedure in a Class II Biological Safety Cabinet or at a laboratory burner is recommended, as is wearing disposable gloves and a suitable lab coat.
- The use of sterile RNase-free pipette filter tips is recommended.
- Avoid cross-transferral of RNA between minicolumns.
- Guanidine salts residues may form highly reactive compounds when combined with oxidation compounds. In case of spillage, clean the surface with a detergent-water solution.
- In case of blood spillage, clean the surface first with detergent water solution and next with 1% sodium hypochlorite.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

Quantity of starting material

When isolating from more than the recommended quantity of starting material, divide the material into several isolations so that each 15 mg (or 10^6 cells) of sample material is isolated with a separate buffer and minicolumn set. If this quantity is exceeded, the homogenizing column may become clogged and/or the isolated RNA may be of low purity. It depends on the type of the tissue, generally the best results of the low volume elution samples (<10 µl) are obtained from the max 5 mg of the tissue.

Sampling and storing the material for RNA isolation

Proper sampling and storing of the biological material prior to RNA isolation is crucial to obtaining a high purity RNA. After sampling, the material should either be preserved by deep freezing at -80°C or in liquid nitrogen or stored in RNase inactivating buffers (e.g. RNA*later*®, Ambion) at -20°C. Most tissues must be preserved within 30 minutes of sampling. Tissues rich in RNases (pancreas, liver) must be preserved immediately.

When isolating from cell cultures, the best results are achieved with fresh material. If storage is unavoidable, discard the supernatant after centrifugation and freeze the cell pellet at -80°C or in liquid nitrogen.

RNase elimination

RNases are very active enzymes which do not require any cofactors and are resistant to autoclaving at 121°C for 15 minutes. In order to avoid the degrading effect of the enzymes on the RNA, the following recommendations should be followed:

a. Use disposable latex, vinyl or nitrile gloves at all times when working with the RNA. Do not touch any items not designed specifically for RNA work.
b. If possible, keep the samples at 2-8°C at all stages of the procedure, including centrifugation. Use decontaminated freezing racks instead of ice in order to avoid RNase contamination. Keeping RNA after elution in the freezing racks is mandatory.
c. Disposable plasticware (tips, tubes) should be RNase-free or autoclaved at 134°C for 18-20 minutes.
d. Reuseable plasticware, glass and porcelain should be soaked overnight in 0.1 N NaOH/0.1% DEPC water (or RNase-free water) and then washed with 0.1% DEPC water (or RNase-free water). When applicable, glass and porcelain (mortars) should be parched at 150-140°C for 2-4 h and cooled to room temperature.

e. Wipe surfaces, pipettes, centrifuge (wipe the rotor separately) and tube racks with 3% hydrogen peroxide or <0.5% sodium hypochlorite (or any other commercially available RNase inactivating fluid). Prior to decontamination, test the decontaminant on a small area of the material for possible undesired reactions.

RNA elution

The optimal volume of the elution buffer REB (RNA Elution Buffer) used should be chosen in line with the amount of the sample material and the final RNA concentration expected. If a high RNA concentration is desired, the elution volume may be reduced down to 5 µl. It is important to notice that in such case the final eluent volume will be reduced each time for about (1-1.5 µl) giving, in case of 5 µl elution, c.a. 3.5-4 µl as a final max eluent volume. It is essential to apply the Elution Buffer precisely onto the centre of the membrane.

If full RNA retrieval is required, a second elution should be performed. For the second elution, repeat steps 13 to 16 of the Isolation Protocol (section XI), placing purification column in a new, sterile 1.5 ml Eppendorf tube.

The Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions.

DNA contamination

All the biological material used for RNA isolation also contains DNA. There is no RNA isolation method which guarantees complete DNA removal unless the RNA sample is treated with DNase after isolation. Even slight DNA contamination (several gDNA copies per reaction) may give an additional signal in a quantitative PCR analysis after reverse transcription. The ***EXTRACTME*** **TOTAL RNA MICRO SPIN KIT** provides efficient on-column digestion of the DNA during RNA purification. The DNase I is removed by a RNA Wash Buffer.

Foam formation in the RLys Buffer

Due to non-ionic detergent content of the lysis buffer, after homogenization, vortexing or intensive pipetting it may create a foam. In order to eliminate the foam, centrifuge at 11k x g for 1 min.

IX. SAMPLE PREPARATION

A. FRESH OR FROZEN SOLID TISSUE

Quantity: up to 15 mg (5 mg optimally)
Sample material: animal or human tissues.

General procedure, applies to all methods of homogenization

Divide tissue into small fragments with tweezers and scissors or scalpel. Follow one of homogenization methods described below or go to step 1 of the Isolation Protocol (section XI).

Liquid nitrogen, dry ice (LN_2, CO_2)

1. Put tissue frozen in LN_2 or CO_2 in a previously chilled, sterile mortar. Using a chilled piston, carefully, but firmly crush the tissue into smaller pieces and then, into a pulp.
2. Transfer the powder thus obtained into a 2 ml tube containing **350 µl RLys Buffer** and go to step 2 of the Isolation Protocol (section XI).
   ⚠ After pulping, a thin, sticky layer may be formed, rather than a powder. If this occurs, add 350 µl RLys Buffer to a mortar and reconstitute the tissue by pipetting and then transfer the lysate into a sterile 2 ml tube. Remember to retrieve the tissue remains from the piston as well.

Homogenization using a mechanical homogenizer equipped with knives

1. Place the tissue in a 2 ml tube, add **100 µl RLys Buffer** and carefully homogenize with a sterile homogenizer tip.
2. After homogenization, retrieve the tissue remains from the knife tip by washing it with **250 µl RLys Buffer**. Combine the fractions thus obtained and transfer the entire volume to a new 2 ml tube.
3. Continue the isolation from step 2 of the Isolation Protocol (section XI).

Homogenization using bead-beating tubes

1. Add **200 µl RLys Buffer** to a 2 ml ceramic bead-beating tube and suspend the sliced tissue in the buffer.
2. Place the tube in the tissue homogenizer and homogenize at 3-4k x g for 30 s. If necessary, repeat the procedure.
   ⚠ If evaluation of the degree of tissue fragmentation is compromised by the foam formation, centrifuge the tube at 11k x g.

⚠ If the tissue homogenizer is not available, the tissue may be homogenized by vortexing using the appropriate 2 ml tube adaptor for at least 5 min at maximum speed.

3. Add **150 µl RLys Buffer** and mix by pipetting.
4. Continue the isolation from step 2 of the Isolation Protocol (section XI).

B. CELL CULTURES

Quantity: up to 10^6 cells

Sample material: cell suspension or adherent cells, fresh or frozen at -80°–196°C.

1. Thaw frozen cells at **37°C**. Centrifuge the cells suspended in growth medium or PBS buffer in a 15 ml falcon tube or a 1.5-2 ml Eppendorf tube at 3k x g. If a compact cell pellet is not formed, wash the cells twice with **1 ml cold PBS buffer**.
2. Add **350 µl RLys Buffer**. Mix thoroughly by vortexing for 30 s and subsequent pipetting.
   ⚠ In some cases when cells tend to form syncytia (myoblasts) or tight connections (epithelial cells) or they are high in number (approx. 10^6 cells), it may be difficult to resuspend them in a RLys Buffer. In such case, pipette carefully, using a ≥1000 µl pipette tip or a sterile syringe. Do not use filter tips.
3. Transfer everything to a new 2 ml tube.
4. Continue the isolation from step 2 of the Isolation Protocol (section XI).

X. BEFORE STARTING

1. Mix well each buffer supplied with the kit. Do not mix the **RLys Buffer** vigorously.
2. Examine the **RLys Buffer**. If a sediment occurred, incubate it at 50°C mixing occasionally until the sediment has dissolved. Cool to room temperature.
3. Prepare freezing rack for storage of the eluted RNA.

XI. ISOLATION PROTOCOL

1. Place the fragmented biological material in a 2 ml tube. Add **350 µl RLys Buffer** and vortex for 60 s.

   ⚠ If a thick foam occurs, centrifuge the sample at 11k x g for 1-2 min. Refer to section VIII. Recommendations and Important Notes.

2. Centrifuge for 2 min at 15-21k x g.

3. Transfer the **supernatant** into an **RNA Homogenizing Column H** placed in a collection tube. Centrifuge for 2 min at 15-21k x g. KEEP the flow-through.

   ⚠ For homogenization using bead-beating tubes: carefully pipet the appropriate volume of the supernatant by placing a 200 µl pipette tip (N.B.: a 1 ml tip may be clogged by the beads) into the filling. Tissue remains should either lie on one side of the tube or at the bottom.

   ⚠ If not all of the supernatant passes through the membrane, repeat the centrifugation for 2 min at ≥21k x g. Should the problem persist, it means that the material was insufficiently homogenized or the digestion time was too short or too much sample material was used for the isolation.

4. Add **350 µl 96% ethanol** to the flow-through. Mix by pipetting or vortexing for 5 s.

5. Transfer **the mixture** thus obtained into an **RNA Purification Micro Spin Column** placed in a collection tube. Centrifuge for 1 min at 15k x g.

   DNA Removal Option:
   a. Prewash the minicolumn with 350 µl RW Buffer and centrifuge for 1 min at 11-15k x g.
   b. For each isolation mix 45 µl 10x DNase I Reaction Buffer and 5 µl reconstituted DNase I. Mix by inverting the tube.
   c. Apply 45 µl mixture onto the center of the RNA Purification Micro Spin Column. Incubate 15 minutes at room temperature. Centrifuge for 20 sec at 11-15k x g and proceed to step 6.

6. Transfer the RNA Purification Micro Spin Column to a new Collection Tube.

7. Add **500 µl RW Buffer** and centrifuge for 1 min at 11-15k x g.
8. Discard the flow-through and reuse the collection tube.
9. Add **500 µl RW Buffer** and centrifuge for 1 min at 11-15k x g.
10. Discard the flow-through and reuse the collection tube.
11. Centrifuge for 1-2 min at 15-21k x g.

    ⚠ The wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from the minicolumn before elution.
12. Discard the collection tube and flow-through and carefully transfer the RNA Purification Micro Spin Column B to a sterile, 1.5 ml Eppendorf microcentrifuge tube.
13. Add **≥5 µl** elution buffer **REB**, precisely, onto the centre of the purification minicolumn membrane.

    ⚠ It is essential to apply the elution buffer precisely onto the centre of the membrane.

    ⚠ Other buffer volumes may be used. For instructions, see to section VIII. Recommendations and important notes.
14. Incubate the minicolumn at room temperature for 3 min.
15. Centrifuge at 7-11k x g for 2 min.
16. Remove the minicolumn and place the tube with the eluted RNA in a freezing rack. The isolated RNA is ready for use in downstream applications or for storage at -80°C.

XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Column H becomes clogged during purification.	Inappropriate tissue homogenization.	Select the appropriate homogenization conditions (see section IXA).
	Tissue and cell remains were transferred into the column H.	Pipette the supernatant carefully, without disturbing the tissue or cell pellet.
	The purification column is overloaded.	See "Column H becomes clogged during purification".
Low RNA isolation efficiency.	Tissue was incorrectly stored or preserved: RNA degradation.	Store tissue at -80°C no longer than a year. If a tissue storage buffer was used, ensure its good quality and that the storage conditions are adequate.
	Too little sample material was used.	Take more sample material.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in the RLys Buffer. The tissue must be first fragmented into as the smallest possible pieces and homogenized by an appropriate method.
	The purification column has become clogged.	See "Column H becomes clogged during purification".
	The RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
Low purified RNA concentration.	Too much of the elution buffer was used.	Decrease the REB volume to ≥5 µl.
Purified RNA is degraded.	Old material was used.	Performing an isolation from fresh tissues is recommended.
	Material was repeatedly frozen/thawed.	Avoid subjecting the sample material to repeated freeze/thaw cycles.
	The RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
	RNA degraded as a result of over-intensive homogenization.	The recommended homogenization conditions should be applied (see section IX).
Low purified RNA concentration.	Too much of the elution buffer was used.	Decrease the REB volume to ≥5 µl.
DNA contamination present.	Too much sample material was used.	Decrease the amount of sample material. Optionally, the purified RNA sample can be treated with a DNase.
	Inappropriate homogenization.	The recommended homogenization conditions should be applied.
	DNase is inactive.	Prepare a fresh DNase solution. Ensure that the DNase solution is stored as recommended.

XIII. SAFETY INFORMATION

RLys Buffer	
	**Hazard** H302+H312+H332, H315, P273, P30+P352, P280, P305+P351+P338, P304+P340
RW Buffer	
	**Hazard** H225, H319, H336 P210, P280, P305+P351+P338

H225 Highly flammable liquid and vapour. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **H336** May cause drowsiness or dizziness. **H302+H312+H332** Harmful if swallowed, in contact with skin and if inhaled. **P210** Keep away from heat/sparks/open flames/hot surfaces. No smoking. **273** Avoid release to the environment. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P305 + P351 + P338 IF IN EYES:** Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.