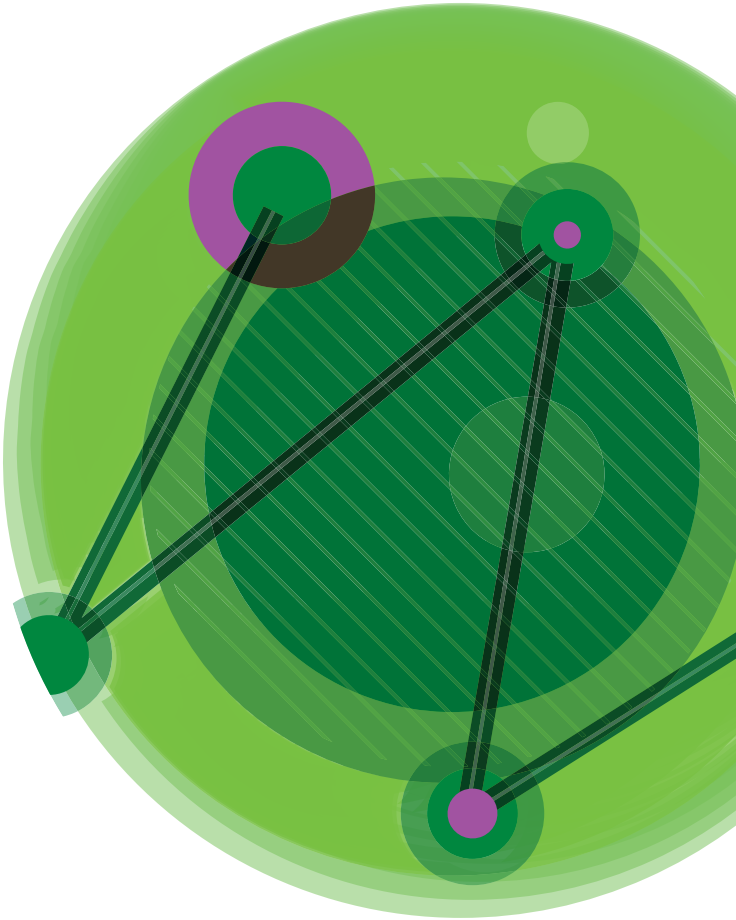
EXTRACTME®
TOTAL RNA MICRO SPIN KIT

Cat. No. EM31.1
Version: 1.2020
NEW VERSION

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



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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	Storage conditions[1]
Catalogue number	EM31.1-010	EM31.1-050	EM31.1-250	
RLys Buffer* (RNA Tissue Lysis Buffer)	3.5 ml	18 ml	88 ml	RT[2]
RW Buffer (RNA Wash Buffer)	13.5 ml	68 ml	338 ml	RT
REB (RNA Elution Buffer)	0.3 ml	1.5 ml	5x 1.5 ml	RT
RNA Homogenizing Columns H	10 pcs	50 pcs	5x 50 pcs	RT
RNA Purification Micro Spin Columns	10 pcs	50 pcs	5x 50 pcs	RT
Collection Tubes (2 ml)	10 pcs	50 pcs	5x 50 pcs	RT

[1] RT – room temperature (+15°C to +25°C)

* 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 β-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 β-mercaptoethanol. Marking the bottle after adding β-mercaptoethanol is recommended.

⚠ Protect RLys Buffer 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.

Expiry date

Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- 96–100% ethanol PFA
- 1.5–2 ml 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:

- DNase I (RNase-free) and Reaction Buffer
- 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. 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 or stored until ready to use.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACTME*** **TOTAL RNA MICRO SPIN KIT** is tested with the use of standard QC procedures. Purified RNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

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

RNA PURITY

A_{260}/A_{280} ratio = 1.9–2.1

VII. SAFETY PRECAUTIONS

- Biological samples should be considered as a biohazardous material on account of its potential pathogen content or health and life-threatening and treated as such substances. While working with tissue and cell cultures, it is essential to follow all safety requirements regarding work with biohazard material.
- It is recommended to carrying out the entire isolation procedure in a Class II Biological Safety Cabinet or at a laboratory burner as well as wearing disposable gloves and a suitable lab coat.
- It is recommended to use sterile RNase-free pipette filter tips.
- Avoid RNA transfer between minicolumns.
- Guanidine salts' residues may form highly reactive compounds when combined with oxidation components. 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 greater than recommended amount of starting material, divide the material into several isolations so each 15 mg (or 10^6 cells) of sample material is isolated with a separate buffer and minicolumn set. Exceeded quantity may clog a purification column and/or lower the purity of isolated RNA. 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 biological material, prior to RNA isolation is crucial to obtain a high purity RNA. After sampling, the material should be preserved by deep freezing (at -80°C or in liquid nitrogen) or stored in RNase inactivating buffers (e.g. RNA*later*®, Ambion). Most tissues should obligatory be preserved within 30 minutes of sampling. Tissues rich in RNases (pancreas, liver) require an immediate preservation.

While isolating from cell cultures, best results are achieved with the use of 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 15 minutes autoclaving at 121°C. In order to avoid enzyme's degrading effect on RNA, it is essential to follow the recommendations:

a. Use disposable gloves at all times when working with RNA. Do not come in contact with any items that are not specifically designed to work with RNA.
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 recommended..
c. Plastic disposables (tips, tubes) should be RNase-free or autoclaved at 134°C for 18–20 minutes.
d. Reuseable plastic, 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 (rotor should be wiped separately) and tube racks with 3% hydrogen peroxide or <0.5% sodium hypochlorite (or any 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 elution buffer REB (RNA Elution Buffer) used should be chosen accordingly to the amount of the sample material and 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 REB 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 RNase-free 1.5 ml Eppendorf tube.

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

DNA contamination

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

Foam formation in the RLys Buffer

Due to detergent content of lysis buffer, after homogenization, vortexing or intensive pipetting it may create a foam. In order to eliminate the foam, centrifuge at 11 000 x g for 60 s.

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 the homogenization methods described below or go to step 1 of 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 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 RNase-free 2 ml tube. Remember to retrieve a 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 obtained and transfer the entire volume to a new 2 ml tube.
3. Continue the isolation from step 2 of 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 a tissue homogenizer and homogenize at 3000–4000 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 11 000 x g.*

⚠ *If the tissue homogenizer is not available, the tissue may be homogenized by vortexing with the use of 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 in a growth medium or PBS buffer in a 15 ml falcon tube or a 1.5–2 ml Eppendorf tube at 400 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. PRIOR TO ISOLATION

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 to store 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 11 000 x g for 60–120s. Refer to section VIII. Recommendations and Important Notes.
2. Centrifuge for 120 s at 15 000–21 000 x g.
3. Transfer the supernatant into an **RNA Homogenizing Column H** placed in a collection tube. Centrifuge for 120 s at 15 000–21 000 x g. KEEP the filtrate.
   - ⚠ 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 ≥ 21 000 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 filtrate. 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 15 000 x g.

   DNA Removal Option:

   a. Prewash the minicolumn with 350 µl RW Buffer and centrifuge for 60 s at 11 000–15 000 x g.
   b. For each isolation mix 45 µl 10x DNase I Reaction Buffer and 5 µl reconstituted DNase I (not included in the kit). 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 s at 11 000–15 000 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 60 s at 11 000–15 000 x g.

8. Discard the filtrate and reuse the collection tube.

9. Add **500 µl RW Buffer** and centrifuge for 60 s at 11 000–15 000 x g.

10. Discard the filtrate and reuse the collection tube.

11. Centrifuge for 60–120 s at 15 000–21 000 x g.

    ⚠ RW Buffer contains alcohol, which may interfere with some enzymatic reactions and decrease the elution efficiency. It is therefore crucial to remove the alcohol completely from the minicolumn before elution.

12. Discard the collection tube and filtrate and carefully transfer the **RNA Purification Micro Spin Column** to a sterile, RNase-free 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 7000–11 000 x g for 120 s.

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 if it was of a good quality and that the storage conditions were adequate.
	Too little sample material was used.	Take more sample material.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in RLys Buffer. The tissue must be first fragmented into smallest possible pieces and homogenized by an appropriate method.
	The purification column has become clogged.	See "Column H becomes clogged during purification".
	RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
Low purified RNA concentration.	Too much of 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.
	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).
DNA contamination present.	Too much sample material was used.	Decrease the amount of a sample material. Optionally, the purified RNA sample may be treated with DNase.
	Inappropriate homogenization.	The recommended homogenization conditions should be applied.
	DNase is inactive.	Prepare fresh DNase solution. Ensure that DNase solution is stored as recommended.

XIII. SAFETY INFORMATION

RLys Buffer

Danger
H331, H302, H412
P261, P264, P301+P312 P330, P304+P340 P311
EUH032

RW Buffer

Danger
H225, H319, H336
P210, P370+P378, P304+P340 P312

EUH032 Contact with acids liberates very toxic gas. **H225** Highly flammable liquid and vapour. **H331** Toxic if inhaled. **H302** Harmful if swallowed. **H319** Causes serious eye irritation. **H336** May cause drowsiness or dizziness. **H412** Harmful to aquatic life with long lasting effects. **P210** Keep away from heat/sparks/open flames/hot surfaces. No smoking. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P370+P378** In case of fire: Use water spray, CO_2, foam, powder; fight larger fires with spray or alcohol resistant foam to extinguish. **P304+P340 P312** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. **P304+P340 P311** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor.