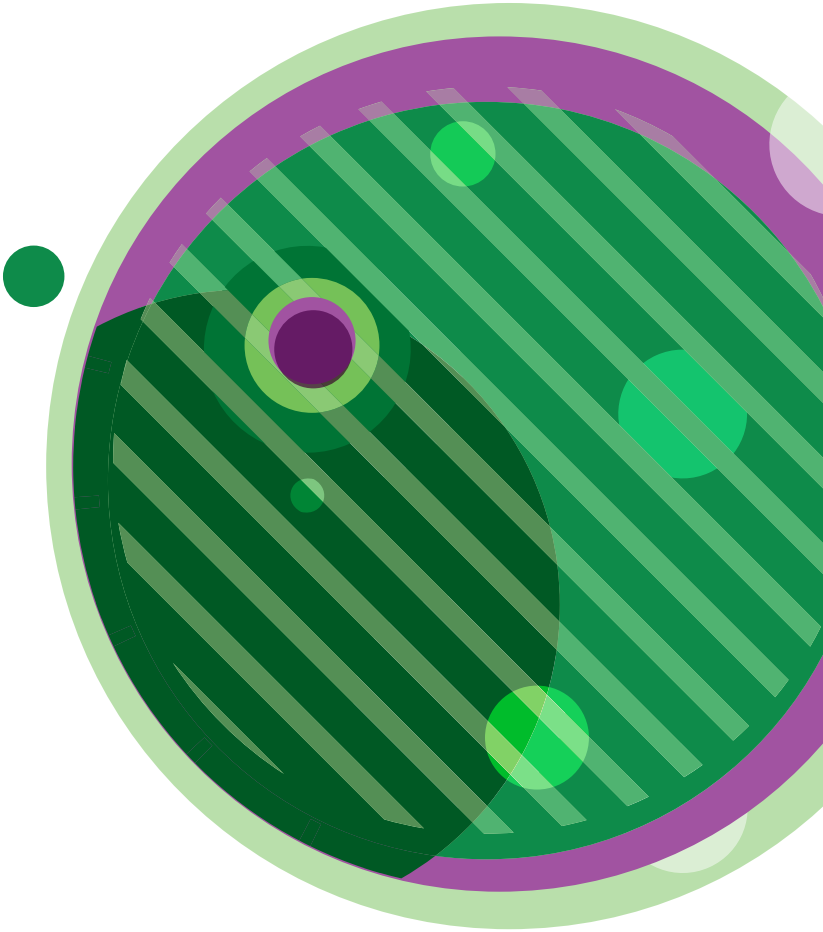
EXTRACTME®
RNA & DNA KIT

Cat. No. EM15
Version: 1.2018

NEW PRODUCT

Kit for simultaneous isolation of RNA & DNA from animal tissue and cell culture



blirt

I. INTENDED USE

The ***EXTRACTME*** **RNA & DNA KIT** is designed for a rapid, simultaneous isolation of high quality total RNA and genomic DNA from a single biological sample. Allows nucleic acids extraction from up to 30 mg of fresh or frozen tissue, and up to 10^7 cultured cells. It is ideal for studying transcriptome (RNA) and genome (DNA) in a single sample. The isolation protocols and buffer formulations were optimized for high isolation efficiency and purity of RNA and DNA. 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	EM15-010	EM15-050	EM15-250	
⚠ **Lys Buffer*** (Lysis Buffer)	6.6 ml	33 ml	165 ml	RT in dark
⚠ **RW1 Buffer** (RNA Wash Buffer 1)	7 ml	35 ml	175 ml	RT in dark
DW1 Buffer** (DNA Wash Buffer 1)	4 ml	19 ml	96 ml	RT
W2 Buffer** (Wash Buffer 2)	5.5 ml	28 ml	2 x 69 ml	RT
REB (RNA Elution Buffer)	1 ml	5 ml	5 x 5 ml	RT
DEB (DNA Elution Buffer)	1 ml	5 ml	5 x 5 ml	RT
RNA Purification Columns	10 pcs	50 pcs	5 x 50 pcs	RT
DNA Purification Columns	10 pcs	50 pcs	5 x 50 pcs	RT
Collection Tubes (2 ml)	2 x 10 pcs	2 x 50 pcs	10 x 50 pcs	RT

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

* For best efficacy during lysis of difficult material and for protection against RNases it is recommended to add **100% β-mercaptoethanol** to **Lys Buffer**, to a **final concentration of 1%**. The combined Lys Buffer and β-mercaptoethanol will remain stable at 2–8°C for a period of four weeks. Therefore, while isolating in parts, transfer the amount of Lys Buffer needed for one experiment to a separate RNase-free bottle/tube and add β-mercaptoethanol. Marking the bottle with added β-mercaptoethanol is recommended.

** Prior to the first use add appropriate amount of **96–100% ethanol** to **DW1 Buffer** and **W2 Buffer**; for details, see the instructions on the bottle label as well as in the table below. DW1 Buffer concentrate should be mixed with ethanol in 1:1 v/v ratio. W2 Buffer concentrate should be mixed with ethanol in 1:4 v/v ratio. Marking the bottle with added alcohol is recommended.

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM15-010	EM15-050	EM15-250
DW1 Buffer	4 ml	19 ml	96 ml
96–100% ethanol	4 ml	19 ml	96 ml
Total volume	8 ml	38 ml	192 ml
W2 Buffer*	5.5 ml	28 ml	2 x 69 ml
96–100% ethanol	22 ml	112 ml	2 x 276 ml
Total volume	27.5 ml	140 ml	2 x 345 ml
OPTIONAL			
Lys Buffer	6.6 ml	33 ml	165 ml
100% β-ME	66 µl	330 µl	1.65 ml

* While isolating RNA without DNase I, diluted W2 Buffer might be prepared in a smaller volume than given in the table. W2 Buffer should be diluted as follows: 1 volume of W2 Buffer to 4 volumes of ethanol. E. g. for 10 isolations without DNase treatment use 2 ml W2 Buffer concentrate and 8 ml 96–100% ethanol.

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

⚠ **Protect the Lys and RW1 Buffers from the sunlight!**

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% and 70% ethanol PFA
- 1.5–2 ml nuclease-free microcentrifuge tubes
- automatic pipettes and pipette tips (nuclease-free)
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 12 000 x g)
- vortex mixer

Might be necessary:

- DNase I (RNase-free)
- scissors, scalpel
- 100% β-mercaptoethanol
- PBS buffer
- freezing racks (< 7°C) for 1.5–2 ml tubes enabling incubation at cooling conditions
- bead-beating tubes with ceramic filling (cat. no. HPLM100, HPLM100a)
- 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
- centrifuge with a rotor for 10–15 ml tubes (cell cultures)
- 3% hydrogen peroxide or < 0.5% sodium hypochlorite

IV. PRINCIPLE

The ***EXTRACTME*** **RNA&DNA KIT** utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids at high concentration of chaotropic salts. During the first isolation step, a tissue is homogenized in order to disintegrate intercellular bonds (epithelial tissue) and fragmentize high-molecular proteins (muscle or connective tissue). A homogenate is lysed with guanidine thiocyanate and detergents. Nucleases are inactivated by guanidine thiocyanate and β-mercaptoethanol (optional). The homogenate is separated from undigested tissue/cell that remains after centrifugation. First, DNA selectively bounds to DNA Purification Column. In the second order, RNA remaining in the filtrate bounds to an RNA Purification Column membrane in the presence of additional ethanol. A three-step washing stage effectively removes impurities and enzyme inhibitors.

Purified RNA and DNA are eluted with the use of low ionic strength buffers and may be used directly in all downstream applications, such as PCR, qPCR, primer extension, sequencing, microarrays, Southern blotting, and in case of RNA also Northern blotting, RT-PCR, cDNA synthesis or stored until ready to use.

V. QUALITY CONTROL

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

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL

- → fresh or frozen tissue: up to 30 mg
- → tissue preserved in RNase inactivating buffers (e.g. RNA*later*®, Ambion): up to 30 mg
- → cell culture: up to 10^7 cells

EFFICIENCY

Typical efficiencies of RNA and DNA isolation from fresh biological material are presented in section XIV.

BINDING CAPACITY

~230 µg RNA, ~60 µg DNA

TIME REQUIRED

- → 10 minutes (for simultaneous RNA/DNA purification, lysis and homogenization time not included)
- → 15–30 minutes for homogenization in liquid nitrogen
- → 15–20 minutes for mechanical homogenization (ceramic beads)

RNA/DNA PURITY

A_{260}/A_{280} ratio = 1.9–2.1

VII. SAFETY PRECAUTIONS

- Tissue is treated as a biohazardous material and treated as such on account of its potential pathogen content or health and life-threatening substances. While working with tissue and cell cultures it is essential to comply with all safety requirements for working with biohazardous material.
- It is advisable to conduct the entire isolation procedure in a Class II Biological Safety Cabinet or at laboratory burner as well as wearing disposable gloves and suitable lab coat at all times.
- Use of sterile pipette filter tips is recommended.
- Avoid the cross-contamination of samples 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 with detergent-water solution and next with 1% sodium hypochlorite.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

Quantity of starting material

While isolating from greater than recommended amount of starting material (> 30 mg, > 10^7 cells), divide the material into several isolations so each 30 mg (or 10^7 cells) portion is isolated with a separate buffer and minicolumn set. Exceeded quantity may clog a purification column and/or lower the purity of isolated nucleic acids. The maximum amount of cells/tissue that can be processed with the ***EXTRACTME*** **RNA&DNA KIT** is dependent on the type of cell line/tissue and its RNA/DNA content. It is recommended to begin with 1x 10^6 cells/ 10 mg if no information regarding your starting material is available. Based on efficiency obtained, the amount of starting material can be increased during next isolation. In case of RNA-rich material, such as liver, it is recommended not to exceed 10 mg per isolation.

Sampling and storing the material for RNA / DNA isolation

Proper sampling and storing of biological material, especially 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 at -20°C 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 below:

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. Reusable plastic, glass and porcelain should be soaked overnight in 0.1 M 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 140–150°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 / DNA elution

The optimal volume of elution buffers REB and DEB (RNA and DNA Elution Buffers) used should be chosen accordingly to the amount of the sample material and final nucleic acid concentration expected. Use of 30–50 µl Elution Buffer is recommended when extracting from up to 10 mg of tissue or up to 10^6 cells; increasing the elution buffer volume to 100 µl is recommended while isolating from 10–30 mg of tissue or 10^6–10^7 cells. When more sample material is to be used for isolation (not recommended as the column may then easily become clogged), full RNA/DNA recovery may be obtained by performing a second elution (100 µl). For the second elution, repeat step 4 of RNA/DNA Purification Protocol (section XIA or B), placing RNA/DNA Purification Column in a new, sterile nuclease-free 1.5 ml Eppendorf tube.

REB and DEB do not contain EDTA, which may interfere with some enzymatic reactions.

RNA / DNA storage and stability

RNA: For a long-term storage keep RNA at -80°C or in liquid nitrogen. The high quality and purity of eluted RNA allows to maintain its integrity during a short-term storage from -20°C to room temperature.
DNA: For a long-term storage keep DNA at -20°C. The high quality and purity of eluted DNA allows to maintain its integrity during a short-term storage from 2–8°C to room temperature.

RNA contamination

Majority of materials contains more RNA than DNA, especially metabolically active tissues, such as glands or epidermis. In case of tissue rich in nucleic acids up to 10 mg of starting material is recommended in order to avoid cross-binding of RNA and DNA.

DNA contamination

In case of tissue rich in nucleic acids, such as liver, up to 10 mg of starting material is recommended in order to avoid cross-binding of RNA and DNA. Some tissues contain very high amounts of DNA (e.g. brain, spleen or thymus) and will overload DNA Purification Column (unless the amount of starting material is very small).

It is worth noting that all biological material used for RNA isolation contains DNA. There is no RNA isolation method that may guarantee a complete DNA removal unless RNA sample is treated with DNase after isolation. In order to obtain RNA suitable for analyses sensitive to trace amounts of DNA, such as RT-qPCR, it is recommended to treat purified RNA with DNase I. ***EXTRACT****ME* **RNA & DNA KIT** also enables efficient on-column digestion of DNA during RNA purification, according to additional protocol (section XII).

IX. SAMPLE PREPARATION

A. FRESH OR FROZEN SOLID TISSUE

Quantity: up to 30 mg (for tissues rich in nucleic acids, such as liver, up to 10 mg is recommended); **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 **600 µl Lys 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 600 µl Lys 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 Lys Buffer** and carefully homogenize with a sterile homogenizer tip.
2. After homogenization, retrieve the tissue remains from the knife tip by washing it with **500 µl Lys Buffer**. Combine the fractions obtained this way 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

We recommend 2 ml bead-beating tubes with ceramic filling for tissue soft homogenization (HPLM100, HPLM500a).

1. Add **600 µl Lys 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 for 30–60 s at 3000–4000 x g. If necessary, repeat the procedure.

   ⚠ *If the tissue homogenizer is not available, the tissue may be homogenized by vortexing with the use of an appropriate 2 ml tube adaptor for at least 5 min at maximum speed.*
3. Continue the isolation from step 2 of Isolation Protocol (section XI).

B. CELL CULTURES

Quantity: up to 10^7 cells
Sample material: cell suspension or adherent cells, fresh or frozen

1. Thaw frozen cells at **37°C**. Centrifuge the cells suspended 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 **600 µl Lys Buffer**. Mix by vortexing.
3. Continue the isolation from step 2 of Isolation Protocol (section XI).

X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit. Do not mix **Lys Buffer** vigorously.
2. Ensure that ethanol has been added to **DW1 and W2 Buffers**. If not, add an appropriate amount of **96–100% ethanol** (volumes can be found on the bottle labels or in the table given in section II).
3. Prepare **70% ethanol** using DEPC-treated water.
4. Examine **Lys**, **RW1** and **DW1 Buffers**. If a sediment occurred in any of them, incubate it at 50°C (**Lys**) or at 37°C (**RW1**, **DW1**) mixing occasionally until the sediment has dissolved. Cool to room temperature.

OPTIONAL:

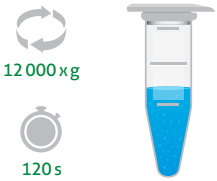
5. Prior to isolation add **100% β-mercaptoethanol** to **Lys Buffer** to a **final concentration of 1%**. **Lys Buffer** after β-mercaptoethanol was added is stable at 2–8°C for 4 weeks. Therefore, while isolating in parts, transfer an appropriate for one isolation amount of Lys Buffer to a separate nuclease-free bottle/tube and add β-mercaptoethanol.
6. Prepare **freezing rack** to store eluted RNA.

XI. ISOLATION PROTOCOL

STEP 1

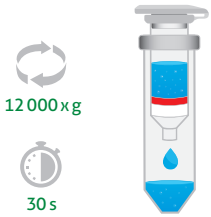
Place a fragmented biological material in a 2 ml tube. Add **600 µl Lys Buffer** and vortex for 60 s.

STEP 2



Centrifuge for 120 s at ≥ 12 000 x g (preferably at 15 000 x g).

STEP 3



Transfer the **supernatant** into **DNA Purification Column (red)** placed in a collection tube and centrifuge for 30 s at ≥ 12 000 x g. **Keep the filtrate**.

Place **DNA Purification Column** in a new **2 ml Collection Tube** for further DNA purification.

⚠ For homogenization with the use of bead-beating tubes: carefully pipet an 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.

⚠ DNA Purification Column can be stored at room temperature for short time period before DNA purification. For longer period store at 2–8°C. Alternatively, DNA purification can be performed simultaneously with RNA purification.

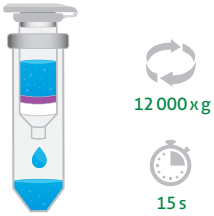
STEP 4

Use the **filtrate** from Step 3 and add **600 µl 70% ethanol**. Mix well by pipetting.

STEP 5

Transfer **up to 700 µl of the obtained mixture** into **RNA Purification Column (violet)** placed in a collection tube. Centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the column together with the collection tube.

Transfer the **remaining mixture** into the same purification minicolumn and centrifuge for 15 s at ≥12 000 x g. Discard the filtrate and place the minicolumn in a new **Collection Tube**.



⚠ To remove trace amount of DNA, an optional on-column DNase I treatment is possible after Step 5. For instructions, go to Section XII.

A. TOTAL RNA PURIFICATION

STEP 1

12 000 x g

15 s

Add **700 µl RW1 Buffer** into **RNA Purification Column (violet)** and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

STEP 2

12 000 x g

15 s

Add **500 µl W2 Buffer** and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

Repeat Step 2.

STEP 3

12 000 x g

90 s

Centrifuge for 90 s at ≥ 12 000 x g (preferably 15 000 x g). Discard the collection tube and the filtrate and carefully transfer the purification minicolumn to a sterile RNase-free 1.5 ml Eppendorf tube.

⚠ W2 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.

STEP 4

12 000 x g

60 s

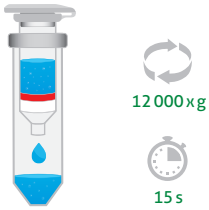
Add **50-100 µl** elution buffer **REB**. Centrifuge for 60 s at ≥ 12 000 x g to elute purified RNA.

⚠ Other buffer volumes in the 30–50 µl range may be used. For instructions, see to section VIII. Recommendations and important notes.

B. GENOMIC DNA PURIFICATION

STEP 1

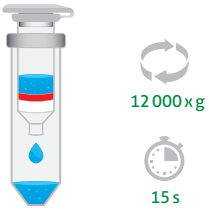
Add **700 µl DW1 Buffer** into **DNA Purification Column (red)** and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.



STEP 2

Add **500 µl W2 Buffer** and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

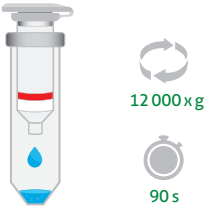
Repeat Step 2.



STEP 3

Centrifuge for 90 s at ≥ 12 000 x g (preferably 15 000 x g). Discard the collection tube and the filtrate and carefully transfer the purification minicolumn to a sterile nuclease-free 1.5 ml Eppendorf tube.

⚠ W2 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.



STEP 4

Add **50-100 µl** elution buffer **DEB**. Centrifuge for 60 s at ≥ 12 000 x g to elute purified DNA.

⚠ Other buffer volumes in the 30–50 µl range may be used. For instructions, see to section VIII. Recommendations and important notes.

XII. OPTIONAL ON-COLUMN DNASE TREATMENT

⚠ This procedure can be performed with a DNase I. For a proper DNase I reconstitution see manufacturer's product manual.

1. Prewash **RNA Purification Column** with **500 µl W2 Buffer** and centrifuge for 60 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.
2. For each isolation mix **90 µl DNase I Reaction Buffer** and **10 µl DNase I (RNase-free) solution** (min 35 U). Mix by inverting the tube.
3. Apply **95 µl mixture** onto the center of **RNA Purification Column**. Incubate for **15 min** at room temperature.
4. Add **600 µl RW1 Buffer** and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube. Proceed to **step 2 of Total RNA Purification Protocol** (Section XIA).

XIII. TROUBLESHOOTING

Problem	Possible cause	Solution
RNA/DNA Purification Column becomes clogged during purification.	Inappropriate tissue homogenization.	Select the appropriate homogenization conditions (see section IXA).
	Tissue and cell debris were transferred into the column.	Pipette the supernatant carefully, without disturbing the tissue or cell pellet.
	The purification column is overloaded.	Do not exceed 30 mg of tissue and 10^7 cells during purification. For tissues rich in nucleic acids, such as liver, do not exceed 10 mg.
Low RNA/DNA yield.	Tissue was incorrectly stored or preserved: nucleic acids degradation.	Store tissue at -80°C no longer than a year. If 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. A proper amount of the material is dependent on the kind of a cell line/tissue examined and needs to be optimized individually.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in Lys Buffer. A tissue must be first fragmented into smallest possible pieces and homogenized by an appropriate method.
	Inefficient homogenization due to an excessive foaming.	Repeat step 2 "Isolation Protocol" section XI.
	The purification column has become clogged.	See "Purification Column becomes clogged during purification".
	RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
	RNA/DNA is still bound to the column membrane.	Repeat the RNA and DNA elution using a proper Elution Buffer.
Low purified RNA/DNA concentration.	Too much of elution buffer was used.	Decrease the REB/DEB volume to 30–50 µl. For a sample concentration it is possible to reload the eluate onto the column and centrifuge again.
Too low A_{260}/A_{230} ratio of purified RNA/DNA.	Remainings of buffers present in the eluate.	Ensure that the purification column had been properly dried before elution and no droplets remained on the ring. If necessary, increase centrifugation speed at step 3 of Total RNA/Genomic DNA Purification Protocol (section XIA or B) to 18 000 x g. Carefully remove the column from a collection tube.
	Incomplete sample loading.	Make sure that lysate has passed completely through the RNA/DNA Purification Column before proceeding through washing steps. If necessary, increase centrifugation speed at steps 3 and 5 of Isolation Protocol (section XI).

Purified RNA / DNA 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 IXA).
RNA / DNA cross-contamination is present.	Too much sample material was used.	Decrease the amount of a sample material.
	Inappropriate homogenization.	The recommended homogenization conditions should be applied (see section IXA).
	High amount of DNA.	The use of on-column DNase treatment is recommended during the RNA purification procedure.
	High amount of RNA.	Decrease the amount of a sample material.

XIV. AVERAGE RNA / DNA ISOLATION EFFICIENCIES FROM FRESH BIOLOGICAL MATERIAL

SAMPLE MATERIAL	Quantity / Mass	RNA Yield	DNA Yield
293 HEK cell line	10^6	5 µg	2 µg
HeLa cell line	10^6	7 µg	3 µg
Liver	5 mg	36 µg	18 µg
Heart	5 mg	10 µg	11 µg
Brain	5 mg	5 µg	16 µg

XV. SAFETY INFORMATION

Lys Buffer

Danger
H302, H331, H412
P261, P271, P304+P340 P311, EUH032

RW1 Buffer

Danger
H225
P210, P303+P361+P353, EUH032

DW1 Buffer

Danger
H315, H318, H412
P280, P305+P351+P338, P310

H225 Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H315** Causes skin irritation. **H318** Causes serious eye damage. **H331** Toxic if inhaled. **H412** Harmful to aquatic life with long-lasting effects. **P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P271** Use only outdoors or in a well-ventilated area. **P280** Wear protective gloves/protective clothing/eye protection/ face protection. **P303+P361+P353** IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower. **P304+P340 P311** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. **P310** Immediately call a POISON CENTER/doctor. **EUH032** Contact with acids liberates very toxic gas.