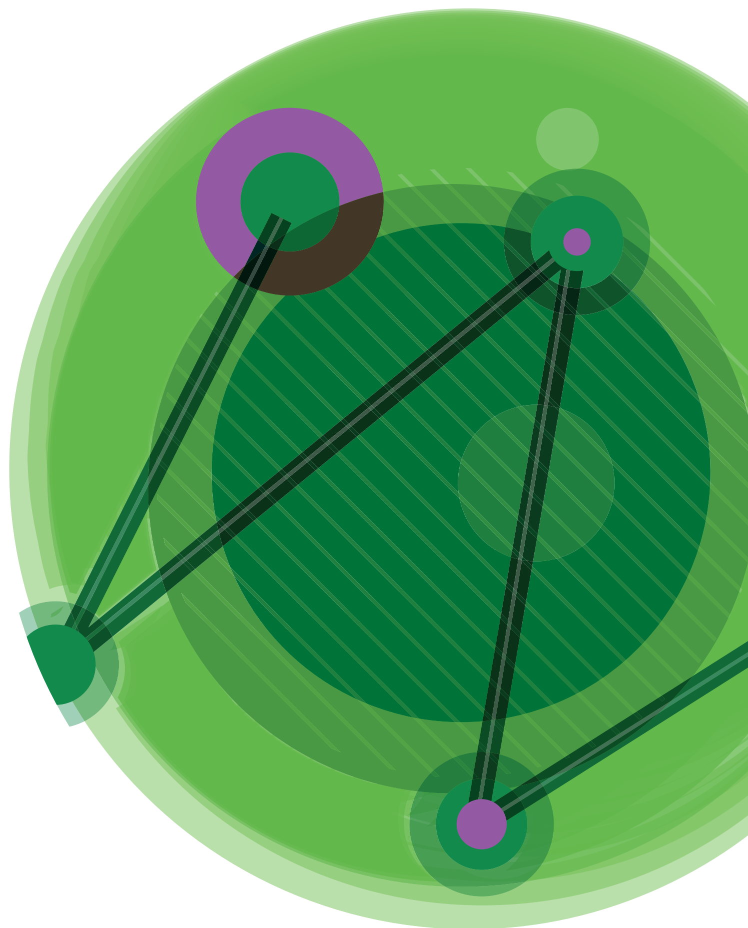
EXTRACTME®
TOTAL RNA KIT

Cat. No. EM09.2, EM11.2
Version: 1.2020
NEW VERSION

Kit for total RNA isolation from animal tissue and cell culture



blirt

EXTRACTME® is a registered trademark of BLIRT S.A.

I. INTENDED USE

The ***EXTRACTME* TOTAL RNA KIT** is designed for a rapid and efficient purification of high quality RNA from up to 30 mg of tissue (fresh or frozen) and up to 10^7 cultured cells. The isolation protocols 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	EM09.2-010	EM09.2-050	EM09.2-250	
Catalogue number*	EM11.2-010*	EM11.2-050*	EM11.2-250*	
⚠ **RLys Buffer**** (RNA 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
RW2 Buffer*** (RNA Wash Buffer 2)	3.3 ml	17 ml	82 ml	RT
REB (RNA Elution Buffer)	1 ml	5 ml	5 x 5 ml	RT
RNA Purification Columns	10 pcs	50 pcs	5 x 50 pcs	RT
Collection Tubes (2 ml)	10 pcs	50 pcs	5 x 50 pcs	RT
Bead-Beating Tubes*	10 pcs	50 pcs	5 x 50 pcs	RT

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

* Refers only to the ***EXTRACTME* TOTAL RNA PLUS KIT**. The Bead-Beating Tubes have ceramic filling.

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

*** Prior to the first use add appropriate amount of **96–100% ethanol** to **RW2 Buffer**; for details, see the instructions on the bottle label as well as in the table below. Marking the bottle with added alcohol is recommended.

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM09.2-010	EM09.2-050	EM09.2-250
Catalogue number*	EM11.2-010*	EM11.2-050*	EM11.2-250*
RW2 Buffer **	3.3 ml	17 ml	82 ml
96–100% ethanol	13.2 ml	68 ml	328 ml
Total volume	16.5 ml	85 ml	410 ml
OPTIONAL			
RLys Buffer	6.6 ml	33 ml	165 ml
100% ß-ME	66 µl	330 µl	1.65 ml

* Refers only to the ***EXTRACTME* TOTAL RNA PLUS KIT**. The Bead-Beating Tubes have ceramic filling.

** RW2 buffer might be prepared in a smaller volume than given in the table. RW2 Buffer should be diluted as follows: 1 volume of RW2 Buffer to 4 volumes of ethanol. E. g. for 10 isolations use 2 ml RW2 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 RLys 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 RNase-free microcentrifuge tubes
- automatic pipettes and pipette tips (RNase-free)
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 12 000 x g)
- vortex mixer

Might be necessary:

- → Antifoam agent
- → DNase I (RNase-free) and Reaction Buffer
- → scissors, scalpel
- → 100% ß-mercaptoethanol
- → 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* TOTAL RNA 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. RNases are inactivated by guanidine thiocyanate and ß-mercaptoethanol (optional). The homogenate is separated from undigested tissue/cell that remains after centrifugation. RNA bounds to a Purification Column membrane by addition of ethanol. Optional step, on-column DNase digestion, enables removal of remaining genomic DNA. A three-step washing stage effectively removes impurities and enzyme inhibitors. Purified RNA is eluted with the use of low ionic strength buffer or RNase-free water and may be used directly in all downstream applications, such as RT-PCR, RT-qPCR, Northern blotting, cDNA synthesis, primer extension, RNA sequencing, microarrays.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACTME*** **TOTAL RNA 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 : 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 isolation from fresh biological material are presented in section XIV.

BINDING CAPACITY

~230 µg RNA

TIME REQUIRED

- → 10–12 minutes (lysis and homogenization time not included)
- → 15–30 minutes for homogenization in liquid nitrogen
- → 15–20 minutes for mechanical homogenization (ceramic beads)
- → 5 minutes for optional DNase I treatment

RNA PURITY

A_{260}/A_{280} ratio = 1.9 – 2.1

VII. SAFETY PRECAUTIONS

- Tissue should be considered 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 follow all safety requirements regarding work with biohazard material.
- It is recommended to carrying out the entire isolation procedure in the 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 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 RNA.

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 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 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. Use of 30–50 µl REB 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 recovery may be obtained by performing a second elution (100 µl). For the second elution, repeat step 9 of Isolation Protocol (section XI), placing the RNA Purification Column in a new, sterile RNase-free 1.5 ml Eppendorf tube.

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

RNA storage and stability

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 contamination

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. Even a slight 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 KIT** allows efficient on-column digestion of DNA during RNA purification as a optional step. DNase I can be removed by RW1 Buffer.

Antifoam RLys Buffer formulation

Due to detergent content of lysis buffer, it may create a foam after homogenization, vortexing or intensive pipetting. In order to avoid this, antifoam agent can be added to RLys Buffer.

RNA clean-up

The ***EXTRACT****ME* **TOTAL RNA KIT** can be used to clean up RNA after enzymatic reactions, such as labeling, RNA ligation or in-solution DNase digestion. RNA sample is mixed with RLys Buffer and ethanol to create conditions that promote selective binding of RNA to the Purification Column. Then, contaminants are efficiently washed away ensuring a high-quality RNA recovery.

IX. SAMPLE PREPARATION

A. FRESH OR FROZEN SOLID TISSUE

Quantity: up to 30 mg / **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 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 600 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.
   Optional: in order to avoid foaming, antifoam agent can be added.
2. After homogenization, retrieve the tissue remains from the knife tip by washing it with **500 µl RLys 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 ***EXTRACTME* TOTAL RNA PLUS KIT** (EM11.2), which already contains tubes pre-filled with ceramic beads.

1. Add **600 µl RLys Buffer** to a 2 ml ceramic bead-beating tube and suspend the sliced tissue in the buffer.
   Optional: in order to avoid foaming, antifoam agent can be added.
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 RLys Buffer** and mix by vortexing.
   Optional: in order to avoid foaming, antifoam agent can be added.
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 **RLys Buffer** vigorously.
2. Ensure that ethanol has been added to **RW2 Buffer**. 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 **RLys** and **RW1 Buffers**. If a sediment occurred in any of them, incubate it at 50°C (**RLys**) or at 37°C (**RW1**) mixing occasionally until the sediment has dissolved. Cool to room temperature.

OPTIONAL:

1. Prior to isolation add **100% ß-mercaptoethanol** to **RLys Buffer** to a **final concentration of 1%**. RLys Buffer after ß-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 RLys Buffer to a separate RNase-free bottle/tube and add ß-mercaptoethanol.
2. Prepare antifoam agent according to the manufacturer's instruction.
3. Prepare DNase I solution according to the manufacturer's instruction.
4. Prepare **freezing rack** to store eluted RNA.

blirt

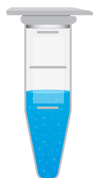
XI. ISOLATION PROTOCOL

STEP 1

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

Optional: antifoam agent can be added.

STEP 2



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

STEP 3

Transfer the supernatant into an **RNase-free 1.5–2 ml** Eppendorf tube and add **600 µl 70% ethanol** to the transferred supernatant. Mix well by pipetting or vortexing.

⚠ 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.

STEP 4

Transfer up to **700 µl of the obtained mixture** into an **RNA Purification Column** 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.

STEP 5 / OPTIONAL (DNase treatment)

a. Prewash the minicolumn with **500 µl RW2 Buffer** and centrifuge for 60 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

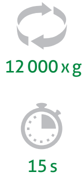
b. For each isolation mix **90 µl Reaction Buffer** and **10 µl reconstituted DNase I** (not included in the kit). Mix by inverting the tube.

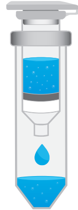
c. Apply **DNase I plus Reaction Buffer** onto the center of the RNA Purification Column. Incubate for 5 minutes at room temperature.

d. 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 7**.

blirt

STEP 6 / omit after DNase treatment





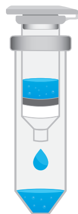
Add **700 µl RW1 Buffer** and centrifuge for 15 s at ≥ 12 000 x g.

Discard the filtrate and reuse the collection tube.

STEP 7

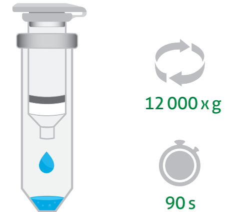
Add **500 µl RW2 Buffer** and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

Repeat step 7.



STEP 8

Centrifuge for 90 s at ≥ 12 000 x g (preferably at 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.

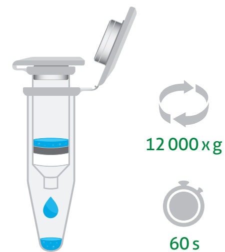


⚠ RW2 Buffer contains alcohol, which may interfere with some enzymatic reactions and decrease the elution efficiency. It is therefore crucial to remove the alcohol completely from the minicolumn before elution.

STEP 9

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

The isolated RNA is ready for use in downstream applications.



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

blirt

XII. RNA CLEAN-UP PROTOCOL

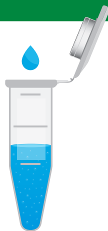
STEP 1

Adjust **RNA sample** volume to **100 µl** by adding **RNase-free water** to a 1.5–2 ml RNase-free Eppendorf tube.

STEP 2

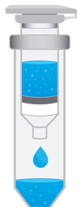
Add **300 µl RLys Buffer**.

STEP 3



Add **300 µl 96-100% ethanol**. Mix well by pipetting or vortexing.

STEP 4



Transfer the mixture into an **RNA Purification Column** placed in a collection tube. Centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

Proceed to **step 6** of Isolation Protocol (section XI).

XIII. TROUBLESHOOTING

Problem	Possible cause	Solution
RNA 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.
Low RNA yield.	Tissue was incorrectly stored or preserved: RNA 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 RLys Buffer. A tissue must be first fragmented into smallest possible pieces and homogenized by an appropriate method.
	Inefficient homogenisation due to an excessive foaming.	Add antifoam agent to RLys Buffer.
	The purification column has become clogged.	See "RNA Purification Column becomes clogged during purification".
	RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
	RNA is still bound to the column membrane.	Repeat the RNA elution.
Low purified RNA concentration.	Too much of elution buffer was used.	Decrease the REB 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.	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 8 of Isolation Protocol (section XI) 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 Purification Column before proceeding through washing steps. If necessary, increase centrifugation speed at step 4 of Isolation Protocol (section XI).

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 IXA).
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.
	High amount of DNA present in a material.	The use of on-column DNA digestion is recommended during the RNA purification procedure. While isolating from high amount of material or particular sample type (e. g. brain), incubation with DNase can be prolonged to 15 minutes.
	Inappropriate homogenization.	The recommended homogenization conditions should be applied (see section IXA).
	DNase is inactive.	Prepare fresh DNase solution. Ensure that DNase solution is stored as recommended.

XIV. AVERAGE RNA ISOLATION EFFICIENCIES FROM FRESH BIOLOGICAL MATERIAL

SAMPLE MATERIAL	Quantity / Mass	Yield
293 HEK cell line	10^6	5 µg
HeLa cell line	10^6	7 µg
Liver	10 mg	75 µg
Heart	5 mg	10 µg
Brain	5 mg	5 µg

XV. SAFETY INFORMATION

RLys Buffer

Danger
H302, H331, H412
P261, P271, P273, P304+P340 P311, EUH032

RW1 Buffer

Danger
H225
P210, P303+P361+P353, EUH032

EUH032 Contact with acids liberates very toxic gas. **H225** Highly flammable liquid and vapour. **H302** Harmful if swallowed. **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. **P273** Avoid release to the environment. **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.

blirt
www.blirt.eu