EXTRACTME®
RNA BACTERIA & YEAST KIT

Cat. No. EM25.1
Version: 1.2020
NEW VERSION

Kit for total RNA isolation from bacteria and yeast

I. INTENDED USE

The ***EXTRACTME*** **RNA BACTERIA & YEAST KIT** is designed for a rapid and efficient purification of high quality RNA from broth yeast or bacteria cultures as well as frozen cells. 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	EM25.1-010	EM25.1-050	EM25.1-250	
RYS Buffer* (RNA Yeast Spheroplast Buffer)	6.6 ml	33 ml	165 ml	RT
⚠ **RYLM Buffer** (RNA Yeast Lysis Mix)	20 µl	100 µl	500 µl	-20°C
⚠ **RNA Extraction Enhancer**	60 µl	300 µl	1.5 ml	-20°C
RYBL Buffer* (RNA Yeast&Bacteria Lysis Buffer)	6.6 ml	33 ml	165 ml	RT in dark
RYBW1 Buffer (conc.)** (RNA Wash Buffer 1)	3.6 ml	18 ml	90 ml	RT in dark
RYBW2 Buffer (RNA Wash Buffer 2)	16.5 ml	83 ml	413 ml	RT
REB (RNA Elution Buffer)	2 ml	10 ml	5x 10 ml	RT
RNA Homogenizing Columns H	10 pcs	50 pcs	5x 50 pcs	RT
RNA Purification Columns B	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)

* Prior to the first use , add **100% ß-mercaptoethanol** to **RYS** and **RYBL Buffers**, to a **final concentration of 1%**. The combined RYS, RYBL Buffer and ß-mercaptoethanol will remain stable at 2–8°C for four weeks. Therefore, when isolating in parts, transfer enough of the RYS, RYBL Buffer for one isolation to a separate RNase-free bottle/tube and add ß-mercaptoethanol. Marking the bottle after adding ß-mercaptoethanol is recommended.

** Prior to the first use , add appropriate amount of **96–100% ethanol** to **RYBW1 Buffer**; for details, see the instructions on the bottle label and in the table below. Marking the bottle after adding the alcohol is recommended.

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS
Catalogue number	EM25.1-010	EM25.1-050	EM25.1-250
RYS Buffer	6.6 ml	33 ml	165 ml
100% ß-mercaptoethanol	66 µl	330 µl	1.65 ml
RYBL Buffer	6.6 ml	33 ml	165 ml
100% ß-mercaptoethanol	66 µl	330 µl	1.65 ml
RYBW1 Buffer	3.6 ml	18 ml	90 ml
96–100% ethanol	3.6 ml	18 ml	90 ml
Total volume	7.2 ml	36 ml	180ml

⚠ Protect the RYBL and RYBW1 Buffers from the sunlight!

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% PFA ethanol (for Wash Buffer RYBW1)
- 70% PFA ethanol (for RNA binding)
- ddH_2O (RNase- and DNase-free) to prepare 70% ethanol
- RNase-free microcentrifuge tubes (1.5–2 ml)
- automatic pipettes and pipette tips (RNase-free)
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 11 000 x g)
- vortex mixer
- freezing racks for 1.5–2 ml tubes
- thermal heating block

May be necessary:

- 100% ß-mercaptoethanol
- DNaseI (RNase-free) nad Reaction Buffer
- lysostaphin 400 U/ml (for RNA isolation from *Staphylococci*)

IV. PRINCIPLE

The ***EXTRACT****ME* **RNA BACTERIA & YEAST KIT** utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids at high concentration of chaotropic salts. In the first step of the isolation procedure the cell walls are disintegrated and the membranes and proteins are degraded. Any RNases are inactivated by guanidine thiocyanate and ß-mercaptoethanol. The homogenate is separated from the undigested cell remains by centrifugation and on the Homogenizing Column. The RNA is bound to the Purification Column membrane by addition of ethanol. The three-step washing stage effectively removes impurities and enzyme inhibitors. Purified RNA is eluted with the use of 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* RNA BACTERIA & YEAST 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

- → broth bacterial (up to 1 x 10^9 cells)
  or yeast (up to 5 x 10^7 cells) culture
- → frozen cell pellet

BINDING CAPACITY

approx. 90 µg RNA

YIELD

It depends on the sample material amount and type.

- → up to 60 µg RNA from bacterial culture
- → up to 30 µg RNA from yeast culture

TIME REQUIRED

- → 30 minutes for RNA isolation from bacteria
  (+ 10 minutes for optional DNase I treatment)
- → 50 minutes for RNA isolation from yeast
  (+ 10 minutes for optional DNase I treatment)

RNA PURITY

A_{260}/A_{280} ratio = 1.9 – 2.1

VII. SAFETY PRECAUTIONS

- Yeast and bacterial cultures are treated as a biohazardous material of its potential pathogen content or health and life-threatening substances. While working with yeasts or bacterial 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 DNA between minicolumns.
- Guanidine salts' residues may form highly reactive components when combined with oxidation compounds. In case of spillage, clean the surface with a detergent-water solution.
- In case of spillage of a liquid containing microorganisms, clean the contaminated surface with a detergent-water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

Sample material

RNA isolation efficiency and purity can depend not only on the number of yeast or bacteria cells, cell type (morphology) or antibiotics in the grow medium, but also on the age and condition of the cells. Extracting RNA from fresh starting material is recommended. Using old or repeatedly frozen/thawed material may result in low efficiency isolation of RNA.

RNase elimination

RNases are very active enzymes which do not require any cofactors and are resistant to autoclaving at 121°C for 20 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 4 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

An optimal volume of Elution Buffer REB (RNA Elution Buffer) used should be chosen in accordance with the quantity of sample material and final RNA concentration expected. If a high RNA concentration is desired, the elution volume may be reduced to 30–50 µl. It should be noted that this may reduce the efficiency of the RNA retrieval. It is essential to apply RNA Elution Buffer precisely to the centre of the membrane. When more sample material is to be used for isolation (not recommended as the column can then easily become clogged), full RNA retrieval can be obtained by performing a second elution (70 µl). For the second elution, repeat steps 18 to 21 of the Isolation Protocol from Bacteria (section XI) or steps 21 to 24 of the Isolation Protocol from Yeast (section XII), placing RNA Purification Column B in a new, sterile 1.5 ml Eppendorf tube.
RNA 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*** **RNA BACTERIA & YEAST KIT** allows efficient on-column digestion of the DNA during RNA purification as a optional step. The DNase I is removed by a RNA Wash Buffer 1.

Foam formation in RYBL Buffer

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

IX. SAMPLE PREPARATION

Isolation from broth culture (0.2–3 ml)

Before starting, always mix broth culture thoroughly. Transfer a desired quantity of yeast or bacterial culture (no more than 1.5 ml) to a sterile 1.5 ml Eppendorf tube and spin down the cells at 3000–4000 x g. Remove the supernatant. While isolating from a greater then 1.5 ml number of cultures, add an extra 1.5 ml of the culture to the top of the cell pellet obtained this way and spin it again. Continue the isolation following the Isolation Protocol from step 2 (section XI – Bacteria or section XII – Yeast).

Isolation from frozen cells

Immediately after retrieving the frozen cell pellet from the freezer continue the isolation following the Isolation Protocol from step 2 (section XI – Bacteria or section XII – Yeast). Do not let the pellet to thaw.

Isolation from Gram-positive bacteria

Gram-positive bacteria must be treated with an appropriate enzyme before commencing the isolation. For RNA isolation from *Staphylococcus* use lysostaphin. For other Gram-positive bacteria, it may be necessary to optimize incubation time or lysozyme concentration.

Staphylococcus:

1. Pellet cells by centrifugation of 1.5 ml of broth culture.
2. Discard the supernatant and suspend the cell pellet in 200 µl TE*.
3. Add **20 µl lysostaphin 400 U/ml** solution and **6 µl RNA Extraction Enhancer**. Mix well by vortexing.
4. Continue the isolation following the Isolation Protocol from step 3 (section XI).

* TE Buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit. Do not mix **RYBL Buffer** vigorously.
2. OPTIONAL: prepare DNase I solution according to the manufacture's instruction.
3. Ensure that ethanol has been added to **RYBW1 Buffer**. If not, add the appropriate amount of **96–100% ethanol** (the volumes can be found on the bottle labels or in the table given in section II).
4. Prior to isolation add **100% ß-mercaptoethanol** to **RYS** and **RYBL Buffers** to **final concentration 1%**. RYS and RYBL Buffers after adding the ß-mercaptoethanol are stable at 2–8°C for 4 weeks. Therefore, when isolating in parts, transfer an appropriate for one isolation amount of RYS or RYBL Buffer to a separate RNase-free bottle/tube and add ß-mercaptoethanol.
5. Examine **RYBL** and **RYBW1 Buffers**. If a sediment occurred in any of them, incubate it at 50°C (**RYBL**) or at 37°C (**RYBW1**) mixing occasionally until the sediment has dissolved. Cool to room temperature.
6. Prepare freezing rack for storage of the eluted RNA.

XI. ISOLATION PROTOCOL FROM BACTERIA

1. Pellet cells from 0.2–1.5 ml bacterial broth culture by centrifugation for 5 min at 3000–4000 x g.
2. Discard the supernatant, and resuspend the cell pellet thoroughly in **100 µl lysosyme** (10 mg/ml in 10 mM Tris – HCl pH 8.0) and **6 µl RNA Extraction Enhancer**.
3. Incubate at **37°C** for 10 min.
4. Add **600 µl RYBL Buffer** and vortex for 60 s.
5. Centrifuge for 120 s at 15 000-21 000 x g.
6. 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.
7. Add **600 µl 70% ethanol** to the filtrate. Mix by pipetting or vortexing for 5 s.
8. Transfer **700 µl of the mixture** obtained this way into an **RNA Purification Column B** placed in a Collection Tube. Centrifuge for 60 s at 15 000 x g. Discard the filtrate and reuse RNA Purification Column B, together with Collection Tube.
9. Transfer the remaining mixture into the same RNA Purification Column B and centrifuge at for 60 s 15 000 x g. Discard the filtrate and place RNA Purification Column B in a new Collection Tube.

   DNA Removal Option:

   a. Prewash RNA Purification Column B with **500 µl RYBW2 Buffer** and centrifuge for 60 s at 11 000–15 000 x g.
   b. For each isolation mix **90 µl 10x DNase I Reaction Buffer** and **10 µl reconstituted DNase I** (not included in the kit). Mix by inverting the tube.
   c. Apply **95 µl mixture** onto the center of RNA Purification Column B. Incubate 15 min at room temperature. Centrifuge for 20 s at 11 000–15 000 x g and proceed to step 10.

10. Add **650 µl RYBW1 Buffer**. Centrifuge for 60 s at 11 000–15 000 x g.
11. Discard the filtrate and reuse Collection Tube.
12. Add **650 µl RYBW2 Buffer**. Centrifuge for 30 s at 11 000–15 000 x g.
13. Discard the filtrate and reuse Collection Tube.
14. Add **500 µl RYBW2 Buffer**. Centrifuge for 60 s at 11 000–15 000 x g.
15. Discard the filtrate and reuse Collection Tube.
16. Centrifuge for 60–120 s at 15 000–21 000 x g.

    ⚠ 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.

17. Discard Collection Tube and filtrate and carefully transfer RNA Purification Column B to a sterile, 1.5 ml Eppendorf microcentrifuge tube.
18. Add **50–100 µl** elution buffer **REB**, precisely, onto the centre of RNA Purification Column B membrane.

    ⚠ Other buffer volumes in the 30–100 µl range may be used. For instructions, see to section VIII. Recommendations and important notes.

19. Incubate RNA Purification Column B at room temperature for 3 min.
20. Centrifuge for 120 s at 7000–11 000 x g.
21. Remove RNA Purification Column B 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. ISOLATION PROTOCOL FROM YEAST

1. Pellet cells from 0.2–1.5 ml yeast broth culture by centrifugation for 5 min at 3000–4000 x g.
2. Discard the supernatant, and resuspend the cell pellet thoroughly in **600 µl RYS Buffer**.
3. Add **2 µl RYLM Buffer**.
4. Incubate at **30°C** for 30 min.
5. After incubation, centrifuge for 10 min at 3000–4000 x g.
6. Carefully, remove the supernatant with a pipette tip. DO NOT disturb the spheroplasts pellet!
7. Add **600 µl RYBL Buffer** and vortex for 60 s.
8. Centrifuge for 120 s at 15 000–21 000 x g.
9. 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.
10. Add **600 µl 70% ethanol** to the filtrate. Mix by pipetting or vortexing for 5 s.
11. Transfer **700 µl of the mixture** obtained this way into an **RNA Purification Column B** placed in a Collection Tube. Centrifuge for 60 s at 15 000 x g. Discard the filtrate and reuse RNA Purification Column B, together with Collection Tube.
12. Transfer the remaining mixture into the same **RNA Purification Column B** and centrifuge at for 60 s 15 000 x g. Discard the filtrate and place RNA Purification Column B in a new Collection Tube.

    DNA Removal Option:

    a. Prewash RNA Purification Column B with **500 µl RYBW2 Buffer** and centrifuge for 60 s at 11 000–15 000 x g.
    b. For each isolation mix **90 µl 10x DNase I Reaction Buffer** and **10 µl reconstituted DNase I** (not included in the kit). Mix by inverting the tube.
    c. Apply **95 µl mixture** onto the center of RNA Purification Column B. Incubate 15 min at room temperature. Centrifuge for 20 s at 11 000–15 000 x g and proceed to step 13.

13. Add **650 µl RYBW1 Buffer**. Centrifuge for 60 s at 11 000–15 000 x g.
14. Discard the filtrate and reuse Collection Tube.
15. Add **650 µl RYBW2 Buffer**. Centrifuge for 30 s at 11 000–15 000 x g.
16. Discard the filtrate and reuse Collection Tube.
17. Add **500 µl RYBW2 Buffer**. Centrifuge for 60 s at 11 000–15 000 x g.
18. Discard the filtrate and reuse Collection Tube.
19. Centrifuge for 60–120 s at 15 000–21 000 x g.

    ⚠ 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.

20. Discard Collection Tube and filtrate and carefully transfer RNA Purification Column B to a sterile, 1.5 ml Eppendorf microcentrifuge tube.
21. Add **50–100 µl** elution buffer **REB**, precisely onto the centre of RNA Purification Column B membrane.

    ⚠ Other buffer volumes in the 30–100 µl range may be used. For instructions, see to section VIII. Recommendations and important notes.

22. Incubate RNA Purification Column B at room temperature for 3 min.
23. Centrifuge for 120 s at 7000–11 000 x g.
24. Remove RNA Purification Column B 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.

XIII. TROUBLESHOOTING

Problem	Possible cause	Solution
Incomplete cell wall lysis.	Too many cells were taken for RNA isolation.	Reduce the amount of starting material.
	No reducing agent added.	Repeat isolation, ensuring that the appropriate quantity of ß-mercaptoethanol has been added.
	BACTERIA: Gram-positive strain was taken for DNA isolation.	Follow the instruction regarding the RNA isolation from Gram-positive bacteria (section IX. Sample preparation).
	YEAST: Reduced efficiency of RNA Yeast Lysis Mix.	Use fresh RYLM Buffer. Ensure that RYLM Buffer is stored at -20°C.
Column H or B becomes clogged during purification.	Incomplete cell lysis.	See "Incomplete cell wall lysis".
	The purification column is overloaded.	Repeat the centrifugation step for 60 s at 21 000 x g or at the maximum speed.
Low RNA isolation efficiency.	Starting material contained few bacterial or yeast cells.	Increase the amount of starting material or decrease the volume of RNA Elution Buffer.
	Incomplete cell lysis.	See "Incomplete cell wall lysis".
	The purification column has become clogged.	See "Column H or B 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 Elution Buffer was used.	Decrease the volume of RNA Elution Buffer to 30-50 µl.

Purified RNA is degraded.	Old material was used.	It is recommended to perform an isolation from fresh overnight bacterial or yeast broth culture.
	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.
Isolated RNA is of poor purity.	One of the washing steps was omitted.	Repeat the isolation, performing both washing steps.
DNA contamination present.	Too much starting material.	Decrease the amount of sample material.
	DNase is inactive.	Prepare a fresh DNase solution. Ensure that the DNase solution is stored as recommended.

XIV. SAFETY INFORMATION

RYBL Buffer

Danger
H331
P261, P304+P340 P311, EUH032

RYBW1 Buffer

Warning
H302, H412
P264, P273, P301+P312 P330, EUH032

RYBW2 Buffer

Danger
H225, H319, H336
P210, P305+P351+P338, P304+P340 P312

EUH032 Contact with acids liberates very toxic gas. **H225** Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H319** Causes serious eye irritation. **H331** Toxic if inhaled. **H336** May cause drowsiness or dizziness. **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. **P264** Wash hands thoroughly after handling. **P273** Avoid release to the environment. **P304+P340 P311** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.