

GeneMATRIX Universal RNA Purification Kit

Universal kit for isolation of total RNA.

● **Cat. no. E3598**

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Content	25 preps E3598-01	100 preps E3598-02	Storage/Stability
LG	6 ml	24 ml	15-25°C
RL	15 ml	60 ml	15-25°C
Wash DN1	12 ml	48 ml	15-25°C
Wash RB1	12 ml	48 ml	15-25°C
Wash RBW	27 ml	108 ml	15-25°C
DNR	1.5 ml	6 ml	15-25°C
RNase-free water	3 ml	12 ml	15-25°C
Homogenization Columns	25	2 x 50	2-8°C
RNA Binding Columns	25	2 x 50	2-8°C
Protocol	1	1	

Introductory Notes

NOTE 1 • Kit Specification. This universal kit is designed for isolation of total RNA (longer than 200 bases) from animal tissues, cell culture, plant material, yeast, any Gram+ and Gram- bacteria and from blood (leukocytes). The kit can also be used for animal tissue RNA purification with Proteinase K digestion, RNA purification with On-Column DNase digestion, or for RNA clean up after molecular biological reactions.

NOTE 2 • Maximum Column Binding Capacity. The total RNA binding capacity is 125 µg per spin-column. The maximum volume of the column reservoir is 600 µl. Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.

NOTE 3 • No DNase Digestion Required. The procedure employs our exclusive homogenization spin-columns, which homogenize sample and removes DNA by binding to the homogenization resin. In addition, washing steps with specifically optimized buffer Wash DN1 effectively eliminates any remaining DNA. However, for RNA applications that are very sensitive to traces of DNA, use Appendix 2 (page 19) with optional on-column DNase digestion.

NOTE 4 • Kit Compounds Storage. Keep all solutions tightly closed to avoid evaporation, resulting in components concentration changes. Store all components of the kit at room temperature apart from homogenization and binding columns, which should be kept in 2-8°C.

NOTE 5 • Maintaining Good Working Practice. To obtain high quality RNA, stick carefully to the protocol provided below. One of the most critical issues during RNA isolation is, to ensure working quickly and with practiced hand. RNA isolation should be performed at room temperature throughout the entire process. Avoid introducing any RNases during the procedure or later handling.

NOTE 6 • β-Mercaptoethanol / DTT. Contaminating RNases are inactivated by addition of reducing agents capable of disrupting disulfide bonds, such as β-mercaptoethanol (β-ME) or dithiothreitol (DTT). To promote reduction of disulfide bonds, add 10 µl β-ME per 1 ml of buffer RL and 10 µl β-ME per 1 ml buffer LG before use. Upon addition of β-ME, RL and LG buffers remains stable for 1 month. A less toxic but more expensive alternative to β-ME is, to add 10 µl of [1 M] DTT in RNase free water per 1 ml buffer RL and per 1 ml buffer LG before use. DTT is not stable in buffers RL and LG, thus DTT-supplemented RL and LG buffers aliquots must not be stored. Working aliquots of [1 M] DTT stock solution in RNase free water must be stored at -20°C for maintaining stability. To set up a [1 M] DTT stock solution (MW = 154.25 g mol⁻¹), dissolve 1.54 g DTT per 10 ml RNase free water and store in aliquots for one-time usage.

Equipment and reagents to be supplied by user:

- For all protocols: ethanol 96–100%, β -mercaptoethanol (14.3 M, β -ME) or [1 M] Dithiothreitol (DTT) in RNase free water, microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5–2 ml tubes, optional **DNase I** for on-column digestion (Cat. no. E1345; see Appendix 2, page 19).
- For bacteria protocol – lysozyme, TE buffer (10 mM Tris pH 7.5, 1 mM EDTA).
- For tissue and plant protocol – equipment for sample disruption and homogenization, depending on the method chosen: mortar and pestle and liquid nitrogen or handheld rotor-stator homogenizer.
- Optional for tissue and plant protocol: antifoaming reagent for EURx lysis buffers **AFR01** (Cat. no. E0328). While using rich in detergents solutions (**LG**) excessive foaming may occur. This is particularly visible when using mechanical homogenizers or when samples are shaken with different types of beads. This foaming is substantially reduced by adding **AFR01** reagent to lysis buffers at a final concentration of 0.5% (v/v) before starting disruption and homogenization (add 5 μ l to 1 ml lysis buffer).
- For tissue protocol – optional **Proteinase K** (Cat. no. E4350; see Appendix 1 page 17).
- For yeast protocol – BeadTubeDry Cat. no E0358 (Appendix 5 page 23), PBS buffer (Cat. no E0281) or 0.9 % NaCl. Optional buffer YL: 1 M sorbitol, 0.1 M EDTA, lyticase (Cat. no. E0329).
- For blood protocol – Lyse RBC buffer (Cat. no. E0326) for erythrocytes lysis. When the blood volume exceeds 400 μ l – appropriate size plastic tubes for erythrocytes lysis and centrifugation after lysis.
- For Appendix 4 (page 21): RNA Purification Protocol with **RNA Extracol** mixture – **RNA Extracol** (Cat. no. E3750), chloroform or 1-bromo-3-chloropropane, isopropanol, ethanol 75%.
- For Appendix 6 (page 24): RNA Purification of plant tissues rich in phenolic compounds and lignins - Lyse Buffer PVP cat. no E0291-01.

Animal Tissue RNA Purification Protocol

NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from animal tissues.

NOTE 2 • For isolation of total RNA from difficult tissues rich in contractile proteins, connective tissue and collagen (for example: heart, muscle or skin tissue) use Appendix 1 (page 17): **RNA Purification Protocol with Proteinase K** digestion. For isolation of total RNA from difficult tissues rich in fat (for example: brain, adipose tissue) use Appendix 4 (page 21): **RNA Purification Protocol with RNA Extracol mixture**.

NOTE 3 • If using mortar and pestle for homogenization sample, do not use more than 30 mg tissues. If using rotor-stator homogenizer use up to 10 times less tissues.

NOTE 4 • Frozen animal tissue should not be allowed to thaw during handling.

NOTE 5 • Add 10 μ l β -mercaptoethanol (β -ME) or 10 μ l DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 800 μ l (2 $\times$ 400 μ l) buffer RL with 8 μ l β -ME. Buffer RL is stable for 1 month after addition of β -ME. DTT is not stable within buffer RL (see page 3, note 6).

1. **a)** Grind animal tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material in RNase-free, cooled 2 ml Eppendorf tube.
 - *To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.*
 - *Frozen tissue should not be allowed to thaw during handling.*
 - *Do not use more than 30 mg tissues.*
- b)** Place the weighed tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 400 μ l buffer **RL**. Homogenize using conventional rotor-stator homogenizer until the sample is homogeneous. Continue the protocol with step 3.
 - *If using mortar and pestle, do not use more than 30 mg tissues. If using rotor-stator homogenizer use up to 10 times less tissues.*
 - *We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.*
2. Add 400 μ l buffer **RL** to a tissue powder. Mix thoroughly by vigorous vortexing.
3. Centrifuge sample for 3 min at maximum speed.
4. Carefully transfer the supernatant to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 $\times$ g for 2 min.
 - *Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.*

5. Add 0.7 volumes of ethanol (96–100%) to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
 - For example, if 400 μ l supernatant was recovered add 280 μ l ethanol.
 - A precipitate may form after addition of ethanol.
6. Apply up to 600 μ l of a mixture to the **RNA binding spin-column (yellow)** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
7. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
8. Add 400 μ l **Wash DN1** buffer and centrifuge at 11 000 x g for 1 min.
 - This step effectively eliminates remaining DNA. However, for RNA applications that are very sensitive to traces of DNA, in the next step use Appendix 2 (page 19) with optional on-column DNase digestion.
9. Remove the spin-column, pour off supernatant and place back into the receiver tube.
10. Add 600 μ l **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
11. Remove the spin-column, pour off supernatant and place back into the receiver tube.
12. Add 300 μ l **Wash RBW** buffer and spin down at 11 000 x g for 2 min.
 - Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.
13. Place spin-column into new receiver tube (1.5–2 ml) and add 30–100 μ l **RNase-free water** directly onto the membrane.
14. Centrifuge for 1 min at 11 000 x g.
15. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and defrosting of RNA).

Cell Culture RNA Purification Protocol

NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from cell culture.

NOTE 2 • Do not use more than 10^6 cells.

NOTE 3 • Add 10 μ l β -mercaptoethanol (β -ME) or 10 μ l DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 800 μ l ($2 \times 400 \mu$ l) buffer RL with 8 μ l β -ME. Buffer RL is stable for 1 month after addition of β -ME. DTT is not stable within buffer RL (see page 3, note 6).

1. Pellet cells by centrifugation in the 2 ml Eppendorf tube for 5 min at 1 000 x g and remove media. Add 400 μ l buffer **RL** to the cell pellet. Pipette the cell lysate several times to ensure sufficient cell disruption.
2. Carefully transfer the lysate to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
 - Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.
3. Add 250 μ l ethanol (96–100%) to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
 - A precipitate may form after addition of ethanol.
4. Apply the sample, including any precipitate, to the **RNA binding spin-column (yellow)** placed in a 2 ml receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.
5. Add 400 μ l **Wash DN1** buffer and centrifuge for 1 min at 11 000 x g.
 - This step effectively eliminates remaining DNA. However, for RNA applications that are very sensitive to traces of DNA, in the next step use Appendix 2 (page 19) with optional on-column DNase digestion.
6. Remove the spin-column, pour off supernatant and place back into the receiver tube.
7. Add 600 μ l **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
8. Remove the spin-column, pour off supernatant and place back into the receiver tube.
9. Add 300 μ l **Wash RBW** buffer and spin down at 11 000 x g for 2 min.
 - Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.

10. Place spin-column into new receiver tube (1.5–2 ml) and add 30–100 µl **RNase-free water** directly onto the membrane.
11. Centrifuge for 1 min at 11 000 x g.
12. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and thawing of RNA).

Plant Tissue RNA Purification Protocol

NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from plant material.

NOTE 2 • If using mortar and pestle, do not use more than 100 mg plant tissues. If using rotor-stator homogenizer use up to 10 times less plant tissues.

NOTE 3 • To buffers RL and LG, respectively, add either 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer aliquot before use. Example: For two RNA preparations, mix 600 µl (2 x 300 µl) buffer RL with 6 µl β-ME or DTT. Buffer RL and LG are stable for 1 month after addition of β-ME. DTT is not stable within buffers RL and LG (see page 3, note 6).

NOTE 4 • In the case of plants with high phenolic compounds level, lignins accumulation or problems with downstream reactions (PCR/ RT-qPCR), it is recommended to exchange LG buffer with Lyse Buffer PVP cat. no E0291-01 and proceed with Appendix 6 (page 24).

1. **a)** Grind plant tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (max. 100 mg) in RNase-free, cooled 2 ml Eppendorf tube. Add 200 µl **LG** buffer and 100 µl **RL** buffer to a plant tissue powder. Mix thoroughly by vigorous vortexing.
 - Do not use more than 100 mg plant tissues.
 - To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.
 - Frozen plant tissue should not be allowed to thaw during handling.
- b)** Place the weighed plant tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 200 µl **LG** buffer and 100 µl **RL** buffer and homogenize using conventional rotor-stator homogenizer until the sample is homogeneous.
 - If using mortar and pestle, do not use more than 100 mg plant tissues. If using rotor-stator homogenizer use up to 10 times less plant tissues.
 - We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.

2. Centrifuge sample for 4 min at maximum speed.
3. Carefully transfer the supernatant to the new, RNase-free, Eppendorf tube and incubate on ice for 10 min.
4. Centrifuge sample for 4 min at maximum speed.
5. Carefully transfer the supernatant to the new, RNase-free, Eppendorf tube and add 200 μ l **RL** buffer. Mix thoroughly by pipetting.
6. Carefully transfer the supernatant to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
 - Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.
7. Add 0.6 volumes of ethanol (96–100%) to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
 - For example, if 400 μ l supernatant was recovered add 240 μ l ethanol.
 - A precipitate may form after addition of ethanol.
8. Apply up to 600 μ l of a mixture to the **RNA binding spin-column (yellow)** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
9. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
10. Add 400 μ l **Wash DN1** buffer and centrifuge for 1 min at 11 000 x g.
 - This step effectively eliminates remaining DNA. However, for RNA applications that are very sensitive to traces amounts of DNA, in the next step use Appendix 2 (page 19) with optional on-column DNase digestion.
11. Remove the spin-column, pour off supernatant and place back into the receiver tube.
12. Add 600 μ l **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
13. Remove the spin-column, pour off supernatant and place back into the receiver tube.
14. Add 300 μ l **Wash RBW** buffer and spin down at 11 000 x g for 2 min.
 - Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.
15. Place spin-column into new receiver tube (1.5–2 ml) and add 30–100 μ l **RNase-free water** directly onto the membrane.
 - It is not necessary to close the tube at this step.

16. Centrifuge for 1 min at 11 000 x g.
17. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and thawing of RNA).

Bacterial RNA Purification Protocol

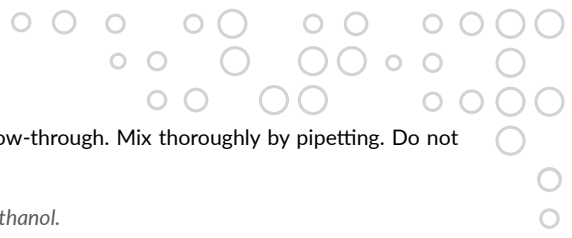
NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from any Gram+ and Gram- bacteria.

NOTE 2 • Prepare TE buffer with 500 µg/ml lysosyme for Gram- bacteria or 5 mg/ml lysozyme for Gram + bacteria.

NOTE 3 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 µl (2 x 350 µl) buffer RL with 7 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, note 6).

NOTE 4 • The bacterial culture should be harvested at 4°C. All subsequent steps of the protocol should be performed at room temperature.

1. Pellet bacteria from overnight culture by centrifugation (for 5 min at 4°C) and discard the supernatant, ensuring that all liquid is completely removed.
 - Do not use more than 1×10^9 bacteria.
 - The highest quality RNA is obtained from bacterial culture, which are either in log phase or early stationary phase.
2. Resuspend the bacterial pellet in 100 µl lysozyme-containing TE buffer (see note 2). Mix by vortexing.
3. Incubate the sample at room temperature for:
 - a) 5–10 min gram-negative bacteria
 - b) 15–20 min gram-positive bacteria
4. Add 350 µl buffer **RL** to the sample. Mix thoroughly by vigorous vortexing.
5. Apply the lysate to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
 - Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.

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6. Add 300 µl ethanol (96–100%) to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
 - A precipitate may form after addition of ethanol.
 7. Apply up to 600 µl of a mixture to the **RNA binding spin-column (yellow)** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 8. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 9. Add 400 µl **Wash DN1** buffer and centrifuge for 1 min at 11 000 x g.
 - This step effectively eliminates remaining DNA. However, for RNA applications that are very sensitive to traces of DNA, in the next step use Appendix 2 (page 19) with optional on-column DNase digestion.
 10. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 11. Add 600 µl **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
 12. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 13. Add 300 µl **Wash RBW** buffer and spin down at 11 000 x g for 2 min.
 - Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.
 14. Place spin-column into new receiver tube (1.5–2 ml) and add 30–100 µl **RNase-free water** directly onto the membrane.
 - It is not necessary to close the tube at this step.
 15. Centrifuge for 1 min at 11 000 x g.
 16. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and thawing of RNA).

Yeast RNA Purification Protocol

NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from yeast.

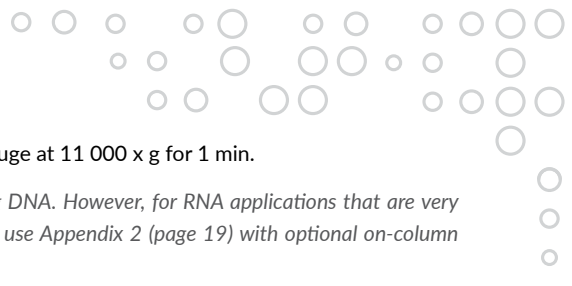
NOTE 2 • We recommend the alternative non-enzymatic method of yeast preparation as described in Appendix 5 (page 23). Mechanical cell wall disruption is more efficient than enzymatic and enables higher yield of RNA.

NOTE 3 • Prepare buffer YL 1 M sorbitol, 0.1 M EDTA. Just before use, add: 10 µl (50 U) lyticase (E0329) per 1×10^7 cells and either 0.1% β-mercaptoethanol or 0.1% DTT.

NOTE 4 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 µl (2 x 350 µl) buffer RL with 7 µl β-ME or DTT. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, note 6).

NOTE 5 • The yeast should be harvested at 2–8°C. After harvesting the cells, all centrifugation steps should be performed at room temperature. Use only freshly harvested cells.

1. Harvest yeast cells by centrifugation at 5 000 x g for 5 min at 2–8°C and discard the supernatant, ensuring that all liquid is completely removed.
 - Do not use more than 5×10^7 yeast cells.
2. Resuspend cells in 1.5 ml lyticase-containing buffer **YL** (see note 2). Incubate for 30 min at 30°C to generate spheroplasts.
 - Use only freshly harvested cells.
3. Pellet the resulting spheroplasts by centrifugation for 5 min at 1 000 x g. Carefully remove the supernatant.
4. Add 350 µl buffer **RL** to lyse pelleted spheroplasts. Mix thoroughly by vigorous vortexing.
5. Apply the lysate to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
 - Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.
6. Add 250 µl ethanol (96–100%) to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
 - A precipitate may form after addition of ethanol.
7. Apply the sample, including any precipitate, to the **RNA binding spin-column (yellow)** placed in a 2 ml receiver tube. Centrifuge for 1 min at 11 000 x g.

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8. Add 400 µl **Wash DN1** buffer and centrifuge at 11 000 x g for 1 min.
 - *This step effectively eliminates remaining DNA. However, for RNA applications that are very sensitive to traces of DNA, in the next step use Appendix 2 (page 19) with optional on-column DNase digestion.*
 9. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 10. Add 600 µl **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
 11. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 12. Add 300 µl **Wash RBW** buffer and spin down at 11 000 x g for 2 min.
 - *Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.*
 13. Place spin-column into the new receiver tube (1.5–2 ml) and add 30–100 µl **RNase-free water** directly onto the membrane.
 14. Centrifuge for 1 min at 11 000 x g.
 15. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and thawing of RNA).

Blood RNA Purification Protocol

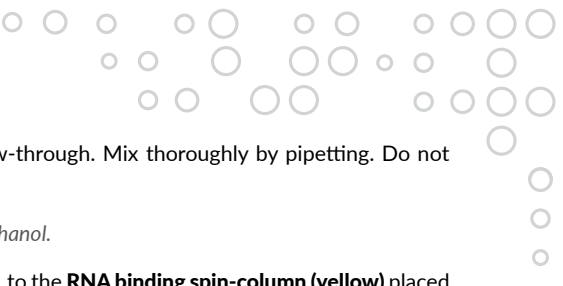
NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from fresh human blood. The kit is not suitable for isolation of RNA from frozen blood. For RNA isolation from stored or frozen blood samples, use the Universal Blood RNA Purification Kit (Cat. no. E3594). In this case the blood must be stabilized with additional buffer included in the kit. After freezing, the blood is not suitable for isolation of RNA using this protocol.

NOTE 2 • One mini column allows purification of RNA with no more than 0.75 ml of blood (up to 10^6 nucleated cells).

NOTE 3 • For RNA isolation from more than 0.75 ml of blood, use appendix 4 (page 21).

NOTE 4 • Add 10 μ l β -mercaptoethanol (β -ME) or 10 μ l DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 μ l (2 x 350 μ l) buffer RL with 7 μ l β -ME or DTT. Buffer RL is stable for 1 month after addition of β -ME. DTT is not stable within buffer RL (see page 3, note 6).

1. Add 4 volumes of buffer Lyse RBC to the fresh blood. Mix by inverting the tube.
 - For example, if the starting blood volume is 300 μ l, add 1200 μ l of Lyse RBC buffer.
 - The maximum amount of blood is 0.75 ml.
 - Do not use frozen blood.
2. Keep at 4°C for 10 min to lyse erythrocytes. Mix twice by inverting the tube.
3. Centrifuge at 400 x g for 10 min at 4°C, and carefully decant the supernatant.
 - Carefully pipette to collect the rest of the supernatant.
4. Add two volumes of Lyse RBC to the leukocytes pellet. Mix thoroughly by vigorous vortexing.
 - For example, if the starting blood volume is 300 μ l, add 600 μ l of Lyse RBC buffer.
5. Centrifuge at 400 x g for 10 min at 4°C, and carefully decant the supernatant.
 - Carefully pipette to collect the rest of the supernatant.
6. Add 400 μ l buffer **RL** to the leukocytes pellet. Mix thoroughly by pipetting for homogenization.
7. Carefully transfer the sample to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
 - Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.

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8. Add 250 µl 96–100% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
 - *A precipitate may form after addition of ethanol.*
 9. Apply the sample, including any precipitate, to the **RNA binding spin-column (yellow)** placed in a 2 ml receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.
 10. Add 400 µl **Wash RB1** buffer and centrifuge at 11 000 x g for 1 min.
 11. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 12. Add 650 µl **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
 13. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 14. Centrifuge at 11 000 x g for additional 1 min to remove residual wash buffer.
 15. Place spin-column into the new receiver tube (1.5–2 ml) and add 30–60 µl **RNase-free water** directly onto the membrane.
 16. Centrifuge for 1 min at 11 000 x g.
 17. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and thawing of RNA).

Fungi RNA Purification Protocol

NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from mushrooms (tissue-like structures) and mycelia, which are free of soil. For RNA/DNA extraction from mycelia with soil (environmental samples) it is recommended to use Environmental DNA & RNA Purification Kit cat. no. E3572.

NOTE 2 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use.

Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, note 6).

NOTE 3 • Do not exceed more than 50 mg of the sample per one RNA preparation. The best sample material is a fresh mushroom or moisty mycelia. Otherwise the purified RNA can be degraded.

NOTE 4 • The sample can be homogenized by mechanical homogenizer, mortar and pestle with liquid nitrogen or Tissue Grinding Tool E0359-03; E0359-04.

NOTE 5 • Tissue Grinding Tool E0359-03 or E0359-04 consists of a 1.5 ml Eppendorf tube containing garnet beads and a pestle for homogenization. The set is available as a separate product.

1. 1. Choose homogenization method:
 - a) Add 400 µl RL buffer to Tissue Grinding Tool, add up to 50 mg of mushroom or mycelia and grind the sample.
 - b) Grind the sample under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material in RNase-free, cooled 2 ml Eppendorf tube.
 - c) Place the sample in a suitably sized vessel for homogenizer. Add 400 µl buffer RL. Homogenize using conventional rotor-stator homogenizer until the sample is homogeneous.

○ *To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder. Frozen tissue should not be allowed to thaw during handling. Do not use more than 30 mg tissues.*
2. In case of homogenization by options a) and c): continue the protocol with step 3. „Animal Tissue RNA Purification Protocol” page 5. In case of homogenization by option b): continue the protocol with step 2. „Animal Tissue RNA Purification Protocol” page 5.

Appendix 1: Animal Tissue RNA Purification Protocol with Proteinase K digestion

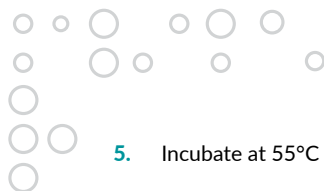
NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from difficult tissues rich in contractile proteins, connective tissue and collagen (for example: heart, muscle or skin tissue).

NOTE 2 • Do not use more than 30 mg tissues. Frozen animal tissue should not be allowed to thaw during handling.

NOTE 3 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 600 µl (2 x 300 µl) buffer RL with 6 µl β-ME or DTT. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, note 6).

NOTE 4 • Prepare Proteinase K (20 mg/ml) solution in water. We recommend using EURx Proteinase K (Cat. no. E4350).

1. **a)** Grind animal tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material in RNase-free, cooled 2 ml Eppendorf tube.
 - *To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.*
 - *Frozen tissue should not be allowed to thaw during handling.*
 - *Do not use more than 30 mg tissues.***b)** Place the weighed tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 300 µl buffer **RL**. Homogenize using conventional rotor-stator homogenizer until the sample is homogeneous. Continue the protocol with step 3.
 - *If using mortar and pestle, do not use more than 30 mg tissues. If using rotor-stator homogenizer use up to 10 times less tissues.*
 - *We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.*
2. Add 300 µl buffer **RL** to a tissue powder. Mix thoroughly by vigorous vortexing.
3. Carefully transfer the sample to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
 - *Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.*
4. Add 500 µl double-distilled water to the flow-through. Mix well and add 15 µl **Proteinase K** solution (20 mg/ml). Mix thoroughly by pipetting.

- 
5. Incubate at 55°C for 10 min.
 6. Add 0.5 volume of ethanol (96–100%) to the sample. Mix thoroughly by pipetting. Do not centrifuge.
 - *A precipitate may form after addition of ethanol.*
 7. Apply 650 µl of the sample, including any precipitate, to the **RNA binding spin-column (yellow)** placed in a 2 ml receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.
 8. Repeat step 7: pipet the remaining sample into the same spin-column, reuse receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.
 9. Add 400 µl **Wash DN1** buffer and centrifuge at 11 000 x g for 1 min.
 - *This step effectively eliminates remaining DNA. However, for RNA applications that are very sensitive to traces of DNA, in the next step use Appendix 2 (page 19) with optional on-column DNase digestion.*
 10. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 11. Add 600 µl **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
 12. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 13. Add 300 µl **Wash RBW** buffer and spin down at 11 000 x g for 2 min.
 - *Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.*
 14. Place spin-column into the new receiver tube (1.5–2 ml) and add 30–60 µl **RNase-free water** directly onto the membrane.
 15. Centrifuge for 1 min at 11 000 x g.
 16. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and thawing of RNA).

Appendix 2: RNA Purification with On-Column DNase digestion

NOTE 1 • Perform this protocol following the washing step with buffer Wash DN1 in the standard protocol, respectively.

NOTE 2 • Perform on-column DNase digestion only by using buffer DNR, which ships with the kit. Other DNase buffers are not compatible with on-column DNase digestion.

NOTE 3 • DNase I is not supplied with this kit, but is available as a separate product (Cat. no. E1345).

NOTE 4 • Prepare DNase I solution before starting this procedure. Add 1–2 U (Kunitz) of DNase I per 50 µl DNR buffer. Do not add more than 2 µl DNase I solution per 50 µl DNR buffer. Dissolve solid DNase I in the storage buffer (50 mM Tris-acetate pH 7.5, 10 mM CaCl₂ and 50% v/v glycerol) in a concentration of 1–2 U/µl (Kunitz) and then add 1 µl DNase I per 50 µl DNR buffer. Use only RNase-free DNase I preparations of high quality.

NOTE 5 • DNase I is sensitive to physical denaturation. Be careful not to mix DNase vigorously.

NOTE 6 • Commercially available RNase-free DNase I preparations vary strongly with respect to their behaviour towards RNA. During routine quality controls, we noted that many commercially available RNase-free DNase I preparations still exhibit residual detectable RNase activity, as visually detectable by band retardations in PAGE gel electrophoresis. Therefore we strongly recommend using EURx RNase-free DNase I (Cat. no. E1345), which does not exhibit residual RNase activity.

1. After the step with **Wash DN1** and centrifugation remove the spin-column, pour off supernatant and place back into the receiver tube.
2. Add 50 µl **DNR** buffer, with **DNase I** added, directly onto the membrane and place on the benchtop at room temperature for 10–20 min. Do not centrifuge.
 - Ensure that DNase I is added to buffer DNR. See note 4 above.
3. Add 400 µl **Wash RB1** buffer and spin down at 11 000 x g for 1 min.
4. Remove the spin-column, pour off supernatant and place back into the receiver tube.
5. Continue with the first **Wash RBW** step in the standard protocol and follow the standard protocol to the end.

Appendix 3: Purification of RNA from molecular biological reactions (e.g. T7 Transcription Assays) or from buffer solutions

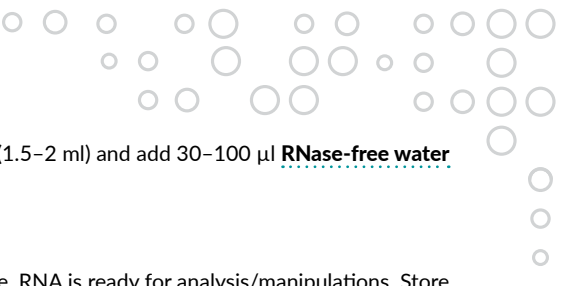
NOTE 1 • This protocol is designed for purification of RNA (longer than 100 bases) from molecular biological reactions (e.g. T7 Transcription Assays EURx E0901) or from buffer solutions.

NOTE 2 • The maximum volume of reaction is 100 µl. The minimum volume of reaction is 30 µl. In case volume of RNA solution is less than 30 µl, add RNase free water to 30 µl total volume.

NOTE 3 • The procedure effectively eliminates remaining DNA.

NOTE 4 • Add either 10 µl β-mercaptoethanol (β-ME) or 10 µl [1 M] DTT per 1 ml buffer RL before use. Example: For two RNA preparations with 40 µl sample volume each, mix 240 µl (2 x 3 volumes) buffer RL with either 2.4 µl β-ME or DTT, respectively.

1. Add 3 volumes of buffer **RL** to 1 volume of reaction sample and mix well by pipetting .
○ For example, add 120 µl buffer RL to a 40 µl reaction sample.
2. Transfer the sample mix to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min. Do not discard the flow-through.
3. Add 0.8 volume of 96% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
○ For example, add 120 µl ethanol to a 150 µl flow-through.
4. Apply max. 600 µl of the sample, including any precipitate, to the **RNA binding spin-column (yellow)** placed in a 2 ml receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.
5. If the sample volume was greater than 600 µl pipet the remaining sample into the same spin-column, reuse receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.
6. Add 400 µl **Wash DN1** buffer and centrifuge at 11 000 x g for 1 min.
7. Remove the spin-column, pour off supernatant and place back into the receiver tube.
8. Add 650 µl **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
9. Remove the spin-column, pour off supernatant and place back into the receiver tube.
10. Centrifuge at 11 000 x g for additional 1 min to remove residual wash buffer. Remove the spin-column, pour off supernatant and place back into the receiver tube.

- 
11. Place spin-column into new receiver tube (1.5–2 ml) and add 30–100 µl **RNAse-free water** directly onto the membrane.
 12. Centrifuge for 1 min at 11 000 x g.
 13. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Store the samples at -20°C or below.

Appendix 4: Animal Tissue RNA Purification Protocol with RNA Extracol mixture

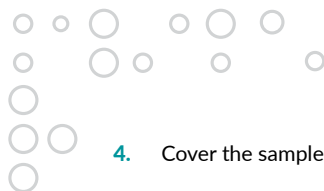
NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) with the use of Phenol-Chloroform mixture from difficult tissues rich in contractile proteins, connective tissue and collagen (for example: heart, muscle or skin tissue) or tissues rich in fat (such as brain or adipose tissue) or tissues deficient in RNA or whole blood samples of more than 0.75 ml volume.

NOTE 2 • The use of phenol-chloroform mixture can significantly increase the amount of initial material, which in the case of difficult tissues or tissues deficient in RNA results in increased concentration of purified RNA in the eluate.

NOTE 3 • Frozen animal tissue should not be allowed to thaw during handling.

NOTE 4 • RNA Extracol is not supplied with this kit, but is available as a separate product (Cat. no. E3700).

1. Homogenize tissue samples in 1 ml of **RNA Extracol** per 10–100 mg of tissue. The tissue can also be homogenized in **RL** buffer (E0310). When using **RL** buffer for homogenization, in the next step, suspension of homogenized tissue must be added to **RNA Extracol** solution. Volume of homogenized tissue should not exceed 10% of the volume of **RNA Extracol** used for RNA isolation.
For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed. Centrifuge sample at 12 000 x g for 10 min at 4°C. Remove and discard the fatty layer.
○ In order to obtain leukocytes pelet from the whole blood samles exceeding 0.75 ml volume, erythrocyte lysis with Lyse RBC buffer needs to be performed according to the instructions from page 14 steps 1-5. Homogenize the leukocytes pelet in RNA Extracol.
2. Incubate samples for 5 min at room temperature.
3. Add 0.2 ml of chloroform (or 0.1 ml 1-bromo-3-chloropropane) per 1 ml of **RNA Extracol** used for homogenization.

- 
4. Cover the sample tightly, shake vigorously for 15 sec.
 5. Incubate samples for 2–5 min at room temperature.
 6. Centrifuge sample at 12 000 x g for 15 min at 4°C.
 - *Centrifugation separates the mixture into 3 phases: violet organic phase, an interphase and a colorless upper aqueous phase (containing RNA). The upper aqueous phase is ~50% of the total volume.*
 7. Remove the aqueous phase very carefully, without disturbing the interphase, and transfer to the new, RNase-free, Eppendorf tube. Add the same volume of ethanol (96–100%). Mix thoroughly. Do not centrifuge.
 - *For example, if 400 µl aqueous phase was collected, add 400 µl ethanol.*
 8. Apply up to 600 µl of the mixture to the **RNA binding spin-column (yellow)** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 9. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 10. Add 400 µl **Wash DN1** buffer and centrifuge at 11 000 x g for 1 min.
 11. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 12. Add 650 µl **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
 13. Centrifuge at 11 000 x g for additional 1 min to remove residual wash buffer.
 14. Place spin-column into new receiver tube (1.5–2 ml) and add 30–100 µl of **RNase-free water** directly onto the membrane to elute bound RNA. Incubate spin-column/receiver tube assembly for 2 min at room temperature.
 15. Centrifuge for 1 min at 11 000 x g.
 16. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and thawing of RNA).

Appendix 5: Alternative yeast RNA isolation using glass beads.

NOTE 1 • This protocol is designed for isolation of total RNA (longer than 200 bases) from yeast.

NOTE 2 • Efficient homogenization of yeast cells is the most crucial step for the high yield and good quality RNA purification. Yeast cell wall can be easily lysed by grinding by glass beads BeadTubeDry Cat. no E0358 in a process of shaking/vortexing. The procedure allows for high yield, enzyme-free RNA purification from yeast.

NOTE 3 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 µl (2 x 350 µl) buffer RL with 7 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, Note 6).

NOTE 4 • GeneMATRIX Universal RNA Purification Kit Cat. no. E3598 does not include BeadTubeDry Cat. no E0358. BeadTubeDry can be purchased separately.

NOTE 5 • The yeast culture should be harvested at 4°C. All subsequent steps of the protocol should be performed at room temperature.

NOTE 6 • Prepare PBS or 0.9 % NaCl solution. PBS buffer can be purchased separately Cat. no E0281.

1. Harvest yeast cells by centrifugation at 5 000 x g for 5 min at 2-8°C and discard the supernatant, ensuring that all liquid is completely removed.
 - Do not use more than 5×10^7 yeast cells.
2. Resuspend cells in 100 µl PBS of 0.9 % NaCl and transfer to the BeadTubeDry.
 - Use only freshly harvested cells.
3. Secure BeadTubeDry horizontally to a vortex by using a vortex adapter or a tube holder. Vortex at maximum speed for 5 min. Do not allow for heating the tubes. After vortexing place the tubes on ice.
 - Cell disruptor (FastPrep, Precellys, Disruptor Genie, etc.) shall preferably be used. Maximum DNA yields are achieved by using a cell disruptor rather than by vortexing. But, for preventing heating the cells, it is required to optimize the shaking time (generally, a time reduction, as compared to the time specified above for vortexing, depending on the specific type of cell disruptor in use).
4. Add 350 µl **RL** into BeadTubeDry and mix by vortexing.

5. Centrifuge sample for 1 min at maximum speed.
6. Carefully transfer the supernatant to the **homogenization spin-column (purple)** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min.
 - *Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.*
7. Continue with the step 6 of the standard Yeast RNA Purification Protocol (page 12).

Appendix 6: RNA Purification of plant tissues rich in phenolic compounds and lignins.

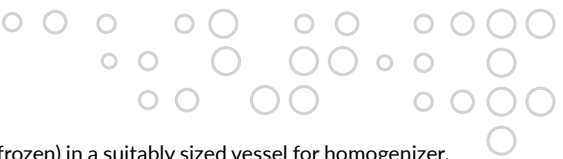
NOTE 1 • In the case of plants with high phenolic compounds level, lignins accumulation or problems with downstream reactions (PCR/ RT-qPCR), it is recommended to exchange LG buffer with Lyse Buffer PVP cat. no E0291-01. Lyse Buffer PVP contains polyvinylpyrrolidone (PVP) actively reducing plant PCR inhibitors and increasing the efficiency of RNA extraction.

NOTE 2 • The protocol is modified by buffer LG exchange to Lyse Buffer PVP followed by samples incubation on ice (step 3).

NOTE 3 • Add either 10 µl β-mercaptoethanol (β-ME) or 10 µl [1 M] DTT per 1 ml Lyse Buffer PVP and RL before use.

NOTE 4 • Lyse Buffer PVP is not supplied with this kit, but is available as a separate product (Cat. no. E0291-01).

1. Choose homogenization method:
 - a) Grind plant tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (max. 100 mg) in RNase_free, cooled 2 ml Eppendorf tube. Add 200 µl Lyse Buffer PVP and 100 µl **RL** buffer to a plant tissue powder. Mix thoroughly by vigorous vortexing.
 - *Do not use more than 100 mg plant tissues.*
 - *To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.*
 - *Frozen plant tissue should not be allowed to thaw during handling.*
 - *Make sure that Lyse Buffer PVP and RL contain β-ME or DTT.*



b) Place the weighed plant tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 200 µl Lyse Buffer PVP and 100 µl **RL** buffer and homogenize using conventional rotor-stator homogenizer until the sample is homogeneous.

○ *If using mortar and pestle, do not use more than 100 mg plant tissues. If using rotor-stator homogenizer use up to 10 times less plant tissues.*

○ *We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.*

2. Centrifuge sample for 4 min at maximum speed and transfer the supernatant into the new, RNase-free, Eppendorf tube.
3. Incubate sample on ice for 10 min.
4. Continue with step 4 page 9.

Safety Information

LG

Warning



H319 Causes serious eye irritation.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/ attention.

RL

Warning



H302+H332 Harmful if swallowed or if inhaled.

H412 Harmful to aquatic life with long lasting effects.

P273 Avoid release to the environment.

P301+P312 If swallowed: call a poison center/ doctor/... if you feel unwell.

P304+P340 If inhaled: remove person to fresh air and keep comfortable for breathing.

EUH032 Contact with acids liberates very toxic gas.

Wash RB1

Warning



H226 Flammable liquid and vapour.

H302 Harmful if swallowed.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P301+P312 If swallowed: call a poison center/ doctor/... if you feel unwell.

P403+P235 Store in a well-ventilated place. Keep cool.

EUH032 Contact with acids liberates very toxic gas.



Wash RBW

Danger



H225 Highly flammable liquid and vapour.

H319 Causes serious eye irritation.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P403+P235 Store in a well-ventilated place. Keep cool.

P337+P313 If eye irritation persists: Get medical advice/ attention.



- ***The GeneMATRIX Universal RNA Purification Kit is designed for rapid, thorough isolation and purification of total RNA from a broad variety of sources: animal, plant, fungi tissues, cell cultures, bacteria and yeast cells or blood (leukocytes), among others.***

Samples are first lysed in the presence of a denaturing buffer. Upon lysis buffer addition, cellular RNases are immediately inactivated. A passage through specifically designed homogenization spin-columns results in shearing of genomic DNA, thus reducing viscosity of the lysate and removing DNA fragments. The flow-through is applied to a binding spin-column with high specificity for RNA. RNA molecules are adsorbed to the matrix and contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water. Typical yields are up to 125 µg total RNA longer than 200 bases. Isolated

RNA is ready for downstream applications like RT-PCR, qRT-PCR, sequencing, NGS, without the need for ethanol precipitation.

Purified RNA is of high purity and integrity (RIN), is DNA-free as well as free of contaminants, such as DNA, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others. Rock-solid GeneMATRIX technology forms the basis for efficient recovery of pure RNA.

- **GeneMATRIX is synonymous for a family of synthetic, new generation, nucleic acid binding membranes. The GeneMATRIX membrane family has gained fame for two striking features: First, for their extraordinary high binding capacity, allowing to isolate nucleic acids with optimal yield.**

Second, for their remarkably high specificity. Even compounds of pronounced chemical similarity such as DNA, RNA and polysaccharides are easily differentiated amongst each other by the selectivity of these highly optimized matrices. This feature allows to isolate highly pure nucleic acids, that remain to work reliable even after being subjected to extended storage periods (years). Or, directly upon isolation, when used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybridization, etc ...

We take great care to fine-tune all components and variables of the nucleic acid purification system towards each other – a multi-parameter optimization. All matrices ship conveniently pre-packed in a ready-to-use spin-column format. Spin columns are

specifically constructed for precise adjustment of liquid flow-through rate to optimal values. Novel binding and washing buffers are developed to take full advantage of GeneMATRIX unique capacity, resulting in isolation of biologically active, high-quality nucleic acids. And, last not least, a lot of time and efforts went into development of the various GeneMATRIXes, thus providing a platform of unique chemical composition.

High and continuous reproducibility of matrix performance is always warranted, since component preparation as well as stringent quality control is entirely performed in-house at EURx Ltd.

Enjoy.



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