GeneMATRIX Universal RNA/miRNA Purification Kit

Universal kit for isolation of total RNA and miRNA from the tissue, plant and cell culture.

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Note: Store homogenization and RNA binding spin-columns at 2÷8°C.

For research use only.
Not for drug, household or other uses.

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Note 1: This kit is designed for isolation of total RNA together with small RNA molecules (15-30 nucleotides) or for isolation only small RNA molecules fraction. **The procedure does not require the use of phenol or chloroform.**
Note 2: The kit is designed to purify RNA/miRNA from a tissue, plant or cell culture.
Note 3: The total RNA binding capacity is 100 µg per spin-column.
Note 4: Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.
Note 5: Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer Lyse ALL before use. Lyse ALL is stable for 1 month after addition of β-ME.
Note 6: Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer RL before use. Buffer RL is stable for 1 month after addition of β-ME.
Note 7: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes. Store the components of the kit at 2÷8ºC.
Note 8: To obtain RNA/miRNA of the highest purity it is important to follow the protocol provided below carefully. During the procedure, work quickly. All steps should be performed at room temperature.

Equipment and reagents to be supplied by user:

1. For all protocols: β-mercaptoethanol (14.3 M, β-ME), ethanol 96-100%, microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5-2 ml tubes.
2. 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.

Protocol

Part I Disruption and lysis of the sample.

Animal tissue

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. Add 200 µl **Lyse ALL** and 300 µl **RL** buffer to a tissue powder. Mix thoroughly by vortexing vigorously.

   b) Place the weighed tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 300 µl **RL** buffer and homogenize using conventional rotor-stator homogenizer until the sample is homogeneous. Add 200 µl **Lyse ALL** to the homogenized sample. Mix thoroughly.

   Note 1: If using mortar and pestle, do not use more than 20 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.
   Note 2 To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.
   Note 3: Frozen tissue should not be allowed to thaw during handling.
   Note 4: Ensure that β-ME is added to Lyse ALL and to RL buffer.

2. Centrifuge sample for 3 min at maximum speed.

3. Depending on needs, go to Part II of the Protocol, or carefully transfer the supernatant to a new 1.5-2 ml Eppendorf-type tube (RNase-free) and proceed to Part III of the Protocol.

Plant

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 **Lyse ALL** and 100 µl **RL** buffer to a plant tissue powder. Mix thoroughly by vortexing vigorously.

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

   Note 1: 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.
   Note 2: To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.
   Note 3: Frozen plant tissue should not be allowed to thaw during handling.
   Note 4: Ensure that β-ME is added to Lyse ALL and to RL buffer.

2. Centrifuge sample for 4 min at maximum speed.

3. Carefully transfer the supernatant to the new, RNase-free, Eppendorf tube and add 0.7 volume of **RL** buffer. Mix thoroughly by pipetting or vortexing vigorously.

   Note 1: For example, if the supernatant volume is 250 µl, add 175 µl RL buffer.

4. Depending on needs, go to Part II or Part III of the Protocol.

Cell culture

1. Centrifuge the cell culture in the 2 ml Eppendorf tube for 5 min at 1000 x g.

   Note 1: Do not use more than $1x10^7$ cells.

2. Add 400 µl buffer **RL** to the cell pellet. Mix thoroughly by vigorous vortexing and pipetting for homogenization.

   Note 2: Ensure that β-ME is added to RL buffer.

3. Add 100 µl **Lyse ALL** to the homogenized sample. Mix thoroughly.

   Note 1: Ensure that β-ME is added to Lyse ALL buffer.

4. Centrifuge sample for 2 min at maximum speed.

5. Depending on needs, go to Part II of the Protocol, or carefully transfer the supernatant to a new 1.5-2 ml Eppendorf-type tube (Rnase-free) and proceed to Part III of the Protocol.

Part II Homogenization, removal of DNA and total RNA binding with miRNA.

1. Carefully transfer the supernatant to the **homogenization spin-column** placed in a 2 ml receiver tube. Centrifuge at maximum speed for 2 minutes.

   Note 1: Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.

2. Add 1.2 volumes of 96-100**%** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, add 480 µl ethanol.
   Note 2: A precipitate may form after addition of ethanol.

3. Apply up to 700 µl of a mixture to the **RNA binding spin-column** and centrifuge at 11000 x g for 1 minute. Remove the spin-column, pour off supernatant and place back into the receiver tube.

4. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11000 x g for 1 minute. Remove the spin-column, pour off supernatant and place back into the receiver tube.

5. Go to Part IV of the Protocol.

Part III Homogenization, removal of DNA and binding of the miRNA only.

1. To the supernatant from the last step of Part I of the Protocol add 0.3 volume of ethanol (**96-100%**). Mix thoroughly. Do not centrifuge.

   Note 1: For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, add 120 µl ethanol.
   Note 2: A precipitate may form after addition of ethanol.

2. Carefully transfer the mixture to the **homogenization spin-column** placed in a 2 ml receiver tube. Centrifuge at maximum speed for 2 minutes.

   Note 1: Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate, removes DNA and macromolecular RNA.

3. Add additional 0.9 volume of 96-100**%** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, in this step add 360 µl ethanol to the flow-through.

4. Apply up to 700 µl of a mixture to the **RNA binding spin-column** and centrifuge at 11000 x g for 1 minute. Remove the spin-column, pour off supernatant and place back into the receiver tube.

5. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11000 x g for 1 minute. Remove the spin-column, pour off supernatant and place back into the receiver tube.

6. Go to Part IV of the Protocol.

Part IV Wash and elution RNA/miRNA.

1. Add 600 µl of **Wash miRNA** buffer to the RNA binding spin-column (from the last step of Part II or Part III of the Protocol) and centrifuge at 11000 x g for 1 minute.
2. Remove the spin-column, pour off supernatant and place back into the receiver tube.
3. Add 600 µl of **Wash miRNA** buffer and spin down at 11000 x g for 1 minute.
4. Remove the spin-column, pour off supernatant and place back into the receiver tube.
5. Centrifuge at 11000 x g for additional 1 minute to remove residual wash buffer.
6. Place spin-column into new receiver tube (1.5-2 ml) and add 40-80 µl RNase-free water directly onto the membrane.

   Note 1: It is not necessary to close the tube at this step.
7. Centrifuge for 2 min at 11000 x g.
8. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Store the samples at -20°C or below.

Appendix 1

Homogenization, removal of DNA and binding separately large RNA and miRNA (using two RNA binding spin-columns).

1. Carefully transfer the supernatant (from Part I of the Universal RNA/miRNA Purification Kit Protocol) to the **homogenization spin-column** placed in a 2 ml receiver tube. Centrifuge at maximum speed for 2 minutes.

   Note 1: Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.

2. Add 0.3 volume of 96-100**%** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, add 120 µl ethanol.
   Note 2: A precipitate may form after addition of ethanol.

3. Carefully transfer the mixture to the **RNA binding spin-column** placed in a 2 ml receiver tube. Centrifuge at maximum speed for 2 minutes.

4. Store the RNA binding spin-column at 2÷8^{o}C for later large RNA purification (part IV of the protocol - Wash and elution RNA/miRNA). **Use the flow-through for miRNA purification.**

   Note 1: This column contains only large RNA.

5. Add additional 0.9 volume of 96-100**%** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, in this step add 360 µl ethanol to the flow-through.

6. Apply up to 700 µl of a mixture to the **new RNA binding spin-column** and centrifuge at 11000 x g for 1 minute. 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 11000 x g for 1 minute. Remove the spin-column, pour off supernatant and place back into the receiver tube.

   Note 1: This column contains only miRNA.

8. Go to Part IV of the Protocol (Wash and elution RNA/miRNA).

Appendix 2:

Purification of RNA/miRNA from molecular biological reactions (e.g. T7 Transcription Assays) or from buffer solutions.

Note 1: This protocol is designed for purification of RNA (and miRNA) 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. When the volume of reaction is below 30 µl add RNase free water to 30 µl.
Note 3: Procedure effectively eliminates remaining DNA.
Note 4: Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer RL before use.

1. Add 3 volume of buffer **RL** to 1 volume of reaction sample and mix well by pipetting .

   Note 1: For example, add 120 µl buffer RL to a 40 µl reaction sample.
   Note 2: Ensure that β-ME is added to buffer RL.

2. Transfer the sample mix to the homogenization spin column placed in a 2 ml receiver tube. Centrifuge at maximum speed for 2 minutes. Do not discard the flow-through.

3. Add 1.2 volume of 96 **%** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: For example, add 120 µl ethanol to a 180 µl flow-through.

4. Apply max. 700 µl of the sample, including any precipitate, to the RNA binding spin-column placed in a 2 ml receiver tube. Centrifuge for 1 min at 11000 x g. Discard the flow-through.

5. If the sample volume was greater than 700 µl pipet the remaining sample into the same spin-column, reuse receiver tube. Centrifuge for 1 min at 11000 x g. Discard the flow-through.

6. Remove the spin-column, pour off supernatant and place back into the receiver tube.

7. Add 650 µl **Wash RBW** buffer and centrifuge at 11000 x g for 1 minute.

8. Remove the spin-column, pour off supernatant and place back into the receiver tube.

9. Centrifuge at 11000 x g for additional 1 minute to remove residual wash buffer.

10. Place spin-column into new receiver tube (1.5-2 ml) and add 40-100 µl RNase-free water directly onto the membrane.

11. Centrifuge for 2 min at 11000 x g.

12. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Store the samples at -20°C or below.

GeneMATRIX is synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures. Novel binding and washing buffers are developed to take full advantage of **GeneMATRIX** capacity, yielding biologically active, high-quality nucleic acids. Matrix is conveniently pre-packed in ready-to-use spin-format. Unique chemical composition of the matrixes along with optimized construction of spin-columns improve the quality of final DNA or RNA preparation. To speed up and simplify isolation procedure, the key buffers are colour coded, which allows monitoring of complete solution mixing and makes purification procedure more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Such DNA or RNA can be directly used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, *in vitro* translation, cDNA synthesis, hybrydization among others. Additional advantage is reproducibility of matrix performance, as component preparation is carried at Eurx Ltd.

GeneMATRIX Universal RNA/miRNA Purification Kit is designed for rapid purification of total RNA enriched with small RNA molecules, shorter than 200 nucleotides (also miRNA ,15-30 nucleotides) or purification small RNA fraction only from animal tissue, plant or cell cultures. Samples are first disrupted, homogenized and lysed in the presence of lysis and denaturing buffers, which inactivates RNases. In the next stage, homogenization spin-columns shear genomic DNA, reducing viscosity of the lysate and eliminating DNA fragments. Addition of ethanol creates the conditions for selective binding of RNA/miRNA to the membrane **GeneMATRIX.** Then sample is applied to a RNA binding spin-column where all RNA molecules are adsorbed to the matrix and contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water. Maximum yields are up to 100 µg total RNA. Isolated RNA/miRNA is ready for downstream applications without the need for ethanol precipitation. **The procedure does not require the use of phenol or chloroform.**