EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Universal RNA / miRNA Purification Kit

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

Cat. no. E3599

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Introductory Notes

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 125 µg per spin-column. The maximum volume of the column reservoir is 650 µl.

NOTE 4 · Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.

NOTE 5 · Contaminating RNases are inactivated by addition of reducing agents capable of disrupting disulfide bonds, such as ß-mercaptoethanol (ß-ME) or dithiothreitol (DTT). To promote reduction of disulfide bonds, add 10 µl ß-ME per 1 ml of buffer RL before use. Upon addition of ß-ME, RL buffer remains stable for 1 month. A less toxic but more expensive alternative to ß-ME is, to add 10 µl of [1 M] DTT in RNase free water per 1 ml buffer RL before use. DTT is not stable in buffer RL, thus DTT-supplemented RL buffer 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.

NOTE 6 · Per 1 ml buffer Lyse ALL, add 10 µl β-mercaptoethanol (β-ME) or [1 M] dithiothreitol (DTT) in RNase free water before use. Lyse ALL is stable for 1 month after addition of β-ME. DTT is not stable in buffer Lyse ALL.

NOTE 7 · All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes. Store the components of the kit at 15–25°C.

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.

Content	25 preps E3599-01	100 preps E3599-02	Storage/Stability
Buffer A	0.9 ml	3.6 ml	15-25°C
Lyse ALL	6 ml	24 ml	15-25°C
RL	15 ml	60 ml	15-25°C
Wash miRNA	30 ml	120 ml	15-25°C
RNase-free water	3 ml	12 ml	15-25°C
Homogenization Columns	25	2 x 50	15-25°C
RNA Binding Columns	25	2 x 50	15-25°C
Protocol	1	1	

Equipment and reagents to be supplied by user

1. For all protocols: either β-mercaptoethanol (14.3 M, β-ME) or [1 M] Dithiothreitol (DTT) in RNase free water, 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.
3. Optional for tissue and plant protocol: antifoaming reagent for EURx lysis buffers **AFR01** (Cat. no. E0328). While using rich in detergents solutions (**Lyse ALL**) 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 µl to 1 ml lysis buffer).

Protocol

Part I Homogenization column activation

1. Apply 30 µl of activation **Buffer A** onto the **homogenization spin-column** (do not spin) and keep it at room temperature till transfering lysate to the spin-column (for best results at least 10 min).

   - *Column activation is not required when performing purification miRNA only (without the large RNA fraction) - part IV of the protocol (page 8).*
   - *Addition of Buffer A onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.*
   - *The membrane activation should be done before starting isolation procedure.*

Part II Disruption and sample lysis

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.

   - *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.*
   - *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.*
   - *Ensure that either β-ME or DTT is added to buffers RL and Lyse ALL (see page 3, notes 5 and 6).*
2. Centrifuge sample for 3 min at maximum speed.
3. Depending on experimental requirements:
   - For isolation of both, miRNA and total RNA, continue with part III of the protocol (page 7).

- For isolation of miRNA only (without the large, total RNA fraction) carefully transfer the supernatant to a new 1.5–2 ml Eppendorf-type tube (RNase-free) and proceed with part IV of the protocol (page 8).
- For separation and fractionation of large RNA from miRNA continue with the steps described in appendix 1 (page 9), Two RNA binding spin-columns are required for recovery of large, total RNA and miRNA within two separate tubes.

Cell culture

1. Centrifuge the cell culture in the 2 ml Eppendorf tube for 5 min at 1000 x g.
   - *Do not use more than $5x10^6$ cells.*
2. Add 400 µl buffer **RL** to the cell pellet. Mix thoroughly by vigorous vortexing and pipetting for homogenization.
   - *Ensure that either β-ME or DTT is added to buffer RL (see page 3, note 5).*
3. Add 100 µl **Lyse ALL** to the homogenized sample. Mix thoroughly.
   - *Ensure that either β-ME or DTT is added to buffer Lyse ALL (see page 3, note 6).*
4. Centrifuge sample for 2 min at maximum speed.
5. Depending on experimental requirements:
   - For isolation of both, miRNA and total RNA, continue with part III of the protocol (page 7).
   - For isolation of miRNA only (without the large, total RNA fraction) carefully transfer the supernatant to a new 1.5–2 ml Eppendorf-type tube (RNase-free) and proceed with part IV of the protocol (page 8).
   - For separation and fractionation of large RNA from miRNA continue with the steps described in appendix 1 (page 9), Two RNA binding spin-columns are required for recovery of large, total RNA and miRNA within two separate tubes.

Plant

1. **a)** Grind plant tissue under liquid nitrogen to a fine powder using a 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.

   - *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.*
   - *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.*
   - *Ensure that either β-ME or DTT is added to buffers RL and Lyse ALL (see page 3, notes 5 and 6).*

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 0.7 volumes of buffer **RL**. Mix thoroughly by pipetting or vortexing vigorously.

   - *For example, if the supernatant volume is 250 µl, add 175 µl RL buffer.*

6. Depending on experimental requirements:

   - For isolation of both, miRNA and total RNA, continue with part III of the protocol (page 7).
   - For isolation of miRNA only (without the large, total RNA fraction) carefully transfer the supernatant to a new 1.5–2 ml Eppendorf-type tube (RNase-free) and proceed with part IV of the protocol (page 8).
   - For separation and fractionation of large RNA from miRNA continue with the steps described in appendix 1 (page 9), Two RNA binding spin-columns are required for recovery of large, total RNA and miRNA within two separate tubes.

Part III Homogenization, DNA removal and total RNA binding with miRNA

1. Carefully transfer the supernatant to the activated **homogenization spin-column** placed in a 2 ml receiver tube. Centrifuge at 12 000 x g for 2 min.

   - *Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.*

2. Add 1.2 volumes of 96–100% [v/v] ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.

   - *For example, if 400 µl supernatant was recovered in last step in part I of the Protocol, add 480 µl ethanol.*

   - *A precipitate may form after addition of ethanol.*

3. Apply up to 600 µl of a mixture to the **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.

4. 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.

5. Continue with part V of the protocol (page 9).

Part IV Homogenization, removal of DNA and binding of miRNA only

1. To the supernatant from the last step of part II of the protocol add 0.6 volumes of ethanol (96–100% [v/v]). Mix thoroughly. Do not centrifuge.

   - *For example, if 400 µl supernatant was recovered in last step in Part I of the protocol, add 240 µl ethanol.*

   - *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 12 000 x g for 2 min.

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

   - *Activation of homogenization spin-column is not necessary in this case.*

3. Add an additional 0.6 volume of 96–100% [v/v] ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.

   - *For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, add in this step 240 µl ethanol to the flow-through.*

4. Apply up to 600 µl of the mixture to the **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.

5. 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.

6. Continue with part V of the protocol (page 9).

Part V Washing and elution of RNA / miRNA

1. Add 500 µl of **Wash miRNA** buffer to the **RNA binding spin-column** (from the last step of either part III or part IV of the protocol) and centrifuge at 11 000 x g for 1 min.

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

3. Add 500 µl of **Wash miRNA** 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. Centrifuge at 11 000 x g for additional 1 min 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.

   - *It is not necessary to close the tube at this step.*

7. Centrifuge for 2 min at 11 000 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. Perform sample specific disruption, lysis and homogenization steps according to part II of the protocol. Carefully transfer the supernatant (from part II of the Universal RNA / miRNA Purification Kit protocol) to the activated **homogenization spin-column** placed in a 2 ml receiver tube. Centrifuge at 12 000 x g for 2 min.

   - *Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.*

2. Add 0.7 volume of 96–100% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.

   - *For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, add 280 µl ethanol.*

   - *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 12 000 x g for 2 min.

4. Store the **RNA binding spin-column** at 2–8°C for later large RNA purification (part V of the protocol — Wash and elution RNA/miRNA). Use the flow-through for miRNA purification.

   - *This column contains only large RNA.*

5. Add additional 0.5 volume of 96–100% [v/v] ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.

   - *For example, if 400 µl supernatant was recovered in last step in Part I of the Protocol, in this step add 200 µl ethanol to the flow-through.*

6. Apply up to 600 µl of a mixture to a new **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.

7. Transfer the remaining mixture to the same **RNA binding spin-column** (as in step 6) and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

   - *This column contains only bound miRNA.*

8. Continue with Part V of the Protocol (Wash and elution RNA/miRNA, page 9).

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 cat. no. 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 either 10 µl β-mercaptoethanol (β-ME) or 10 µl [1 M] DTT in RNase free water per 1 ml buffer RL before use (see page 3, note 5).

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.*
   - *Ensure that either β-ME or DTT is added to buffer RL.*
2. Transfer the sample mix to the activated **homogenization spin-column** placed in a 2 ml receiver tube. Centrifuge at 11 000 x g for 2 min. Do not discard the flow-through.
3. Add 1.1 volume of 96% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.
   - *For example, add 165 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** 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. Remove the spin-column, pour off supernatant and place back into the receiver tube.
7. Add 600 µl **Wash miRNA** 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.
8. Add 400 µl **Wash miRNA** 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.

9. Centrifuge at 11 000 x g for additional 1 min 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 1 min at 11 000 x g.
12. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Store the samples at -20°C or below.

Safety Information

Buffer A

Danger

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331 If swallowed: Rinse mouth. Do not induce vomiting.
P303+P361+P353 If on skin (or hair): take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a poison center/doctor.
P405 Store locked up.

Lyse ALL

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 miRNA

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.

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

				ISOLATION OF RNA					
				E3700	E3594	E3596	E3598	E3599	E3593
				RNA EXTRACOL [2]	UNIVERSAL BLOOD RNA	HUMAN BLOOD RNA	UNIVERSAL RNA	UNIVERSAL RNA /miRNA	FFPE RNA Purification Kit
				PREPS					
				25 100	25	25	25 100	25 100	25 100
RNA	TOTAL RNA LONGER THAN 200 BASES	ANIMAL TISSUE					●	●	
		PLANT TISSUE					●	●	
		BACTERIA					●		
		YEAST					●		
		CELL CULTURE					●	●	
		HUMAN BLOOD	FRESH	●	●	●	●		
			FROZEN [1]		●				
		ANIMAL BLOOD	FRESH	●	●				
			FROZEN [1]		●				
	miRNA OR TOTAL RNA	ANIMAL TISSUE		●				●	
		FFPE TISSUE SECTIONS							●
		PLANT TISSUE		●				●	
		CELL CULTURE		●				●	
		BACTERIA		●					
		YEAST		●					
		BLOOD/LEUKOCYTES		●					
	PURIFICATION OF RNA AFTER ENZYMATIC REACTIONS						●	●	
	ON-COLUMN DNase DIGESTION				●		●		

All kits contain buffers WASH in ready to use form

1. Frozen with the addition of Lyse Blood buffer (included in kit).
2. Phenol-based reagent for isolation RNA.

GeneMATRIX is a 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.**

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