GeneMATRIX Universal RNA Purification Kit

Universal kit for isolation of total RNA.

Cat. no. E3598

Version 2.3

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

EURx Ltd. 80-297 Gdansk Poland ul. Przyrodnikow 3, NIP 957-07-05-191, KRS 0000202039,
www.eurx.com.pl orders: *email*: orders@eurx.com.pl *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

	SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL	RNA												DNA/RNA/PROTEIN FROM THE SAME BIOLOGICAL SAMPLE					DNA																				
		TOTAL RNA LONGER THAN 200 BASES									miRNA								GENOMIC																	PLASMID			
		ANIMAL TISSUE	PLANT TISSUE	BAKTERIA	YEAST	CELL CULTURE	HUMAN BLOOD		ANIMAL BLOOD		ANIMAL TISSUE	PLANTS	CELL CULTURE	ANIMAL TISSUE	PLANTS	BAKTERIA	YEAST	CELL CULTURE	BAKTERIA	YEAST	CELL CULTURE	PLANTS AND MUSHROOMS	FRESH AND FROZEN BLOOD	SOIL	STOOL	SWAB	SOLID TISSUES	LIQUID TISSUES	RODENT TAILS	HAIR	INSECTS	URINE	BONE	BIOLOGICAL TRACES	FOOD	BAKTERIA	YEAST	ISOLATION FROM AGAROSE GELS	PURIFICATION OF PCR PRODUCTS/DNA AFTER ENZYMATIC REACTIONS
							FRESH	FROZEN	FRESH	FROZEN																													
E3540	AGAROSE																																					X	
E3580	BACTERIAL & YEAST GENOMIC																		X	X																	X		
E3545	BASIC																																			X		X	X
E3510	BIO – TRACE																																	X					
E3560	BONE																																X						
E3555	CELL CULTURE																				X																		
E3525	FOOD																																		X				
E3520	PCR / DNA CLEAN-UP																																						X
E3595	PLANT & FUNGI																					X																	
E3500	PLASMID MINIPREP																																			X			
E3565	QUICK BLOOD																						X																
E3515	SHORT / DNA CLEAN-UP																																						X
E3570	SOIL																							X															
E3575	STOOL																								X														
E3530	SWAB EXTRACT																									X													
E3550	TISSUE																				X						X	X	X	X	X	X							
E3551	TISSUE & BACTERIAL																		X		X						X	X	X	X	X	X							
E3600	MICELLULA DNA																																						X
E3596	HUMAN BLOOD RNA						X																																
E3594	UNIVERSAL BLOOD RNA						X	X	X	X																													
E3597	UNIVERSAL DNA/RNA /PROTEIN													X	X	X	X	X																					
E3598	UNIVERSAL RNA	X	X	X	X	X																																	X
E3599	UNIVERSAL RNA /miRNA	X	X			X					X	X	X																										X

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Introductory Notes

Equipment and reagents to be supplied by user:

1. For all protocols: β-mercaptoethanol (14.3 M, β-ME), 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 16).
2. For bacteria protocol – lysosyme, TE buffer (10 mM Tris pH 7.5, 1 mM EDTA), ethanol 96-100 %.
3. 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.
4. For tissue protocol - ethanol 70 %, optional Proteinase K and ethanol 96-100 % (see Appendix 1 page 14)
5. For plant protocol - ethanol 96-100 %.
6. For yeast protocol – buffer YL: 1 M sorbitol, 0.1 M EDTA, lyticase/zymolase, ethanol 70 %.
7. For cell culture protocol - ethanol 70 %.

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 reach in contractile proteins, connective tissue and collagen (for example: heart, muscle or skin tissue) use Appendix 1 (page 14): **RNA Purification Protocol with Proteinase K digestion.**
Note 3: The RNA binding capacity is 100 µg per minicolumn.
Note 4: 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.
Note 5: Frozen animal tissue should not be allowed to thaw during handling.
Note 6: Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer RL before use. Example: For two RNA preparations, mix 800 µl (2 x 400 µl) buffer RL with 8 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME.
Note 7: Avoid overloading the minicolumn. Overloading will significantly reduce yield and purity and may block the minicolumn.
Note 8: Avoid introducing any RNases during the procedure or later handling.
Note 9: 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 16) with optional on-column DNase digestion.
Note 10: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
Note 11: To obtain RNA 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.

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.

   Note 1: To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.
   Note 2: Frozen tissue should not be allowed to thaw during handling.
   Note 3: 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 µl buffer **RL.** Homogenize using conventional rotor-stator homogenizer until the sample is homogeneous. Continue the protocol with step 3.

   Note 1: 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.
   Note 2: We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.

2. Add 400 µl buffer **RL** to a tissue powder. Mix thoroughly by vigorous vortexing.

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

3. Centrifuge sample for 3 min at maximum speed.

4. 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.

5. Add 350 µl **70 %** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: A precipitate may form after addition of ethanol.

6. Apply 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.

7. Add 400 µl **Wash DN1** buffer and centrifuge at 11,000 x g for 1 minute.

Note 1: 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 16) with optional on-column DNase digestion.

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

9. Add 650 µl **Wash RBW** buffer and centrifuge at 11,000 x g for 1 minute.

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

11. Add 350 µl **Wash RBW** buffer and spin down at 11,000 x g for 2 minutes.

Note 1: 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 minute.

12. Place spin-column into new receiver tube (1.5-2 ml) and add 40-60 µl RNase-free water directly onto the membrane.

Note 1: It is not necessary to close the tube at this step.

13. Centrifuge for 1 min at 11,000 x g.

14. 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: The RNA binding capacity is 100 µg per minicolumn.
Note 3: Do not use more than $1x10^7$ cells.
Note 4: Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer RL before use. Example: For two RNA preparations, mix 800 µl (2 x 400 µl) buffer RL with 8 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME.
Note 5: Avoid overloading the minicolumn. Overloading will significantly reduce yield and purity and may block the minicolumn.
Note 6: Avoid introducing any RNases during the procedure or later handling.
Note 7: 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 16) with optional on-column DNase digestion.
Note 8: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
Note 9: To obtain RNA 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.

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.

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

3. Carefully transfer the lysate 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.

4. Add 350 µl **70 %** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: A precipitate may form after addition of ethanol.

5. Apply 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.

6. Add 400 µl **Wash DN1** buffer and centrifuge for 1 minute at 11,000 x g (approx. 12,000 rpm in standard laboratory centrifuges).

   Note 1: 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 16) with optional on-column DNase digestion.

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 minute.

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

10. Add 350 µl **Wash RBW** buffer and spin down at 11,000 x g for 2 minutes.

Note 1: 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 minute.

11. Place spin-column into new receiver tube (1.5-2 ml) and add 40-60 µl RNase-free water directly onto the membrane.

Note 1: It is not necessary to close the tube at this step.

12. Centrifuge for 1 min at 11,000 x g.

13. 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).

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: The RNA binding capacity is 100 µg per minicolumn.
Note 3: 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 4: Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer RL or buffer LG before use. Example: For two RNA preparations, mix 600 µl (2 x 300 µl) buffer RL with 6 µl β-ME. Buffers are stable for 1 month after addition of β-ME.
Note 5: Avoid overloading the minicolumn. Overloading will significantly reduce yield and purity and may block the minicolumn.
Note 6: Avoid introducing any RNases during the procedure or later handling.
Note 7: 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 16) with optional on-column DNase digestion.
Note 8: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
Note 9: To obtain RNA 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.

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.

   Note 1: Do not use more than 100 mg plant tissues.
   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 LG and to RL buffers.

   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.

   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.
   Note 2: Ensure that β-ME is added to LG and to RL buffers.
   Note 3: 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 add 200 **µl RL** buffer. Mix thoroughly by pipetting.

4. 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.

5. Add 300 µl ethanol (**96-100 %**) to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

Note 1: A precipitate may form after addition of ethanol.

6. Apply 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.

7. Add 400 µl **Wash DN1** buffer and centrifuge for 1 minute at 11000 x g (approx. 12,000 rpm in standard laboratory centrifuges).

Note 1: 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 16) with optional on-column DNase digestion.

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

9. Add 650 µl **Wash RBW** buffer and centrifuge at 11,000 x g for 1 minute.

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

11. Add 350 µl **Wash RBW** buffer and spin down at 11,000 x g for 2 minutes.

Note 1: 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 minute.

12. Place spin-column into new receiver tube (1.5-2 ml) and add 40-60 µl RNase-free water directly onto the membrane.

Note 1: It is not necessary to close the tube at this step.

13. Centrifuge for 1 min at 11,000 x g.

14. 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).

BACTERIAL RNA Purification Protocol

Note 1: This protocol is designed for isolation of total RNA (longer than 200 bases) from any a Gram $^+$ and Gram$^-$ bacteria.
Note 2: The RNA binding capacity is 100 µg per minicolumn.
Note 3: Prepare TE buffer with 500 µg/ml lysosyme for Gram$^-$ bacteria or 5 mg/ml lysosyme for Gram $^+$ bacteria.
Note 4: Add 10 µl β-mercaptoethanol (β-ME) 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.
Note 5: Avoid overloading the minicolumn. Overloading will significantly reduce yield and purity and may block the minicolumn.
Note 6: To obtain RNA of the highest purity it is important to follow the protocol provided below carefully. During the procedure, work quickly. The bacterial culture should be harvested at 4°C. All subsequent steps of the protocol should be performed at room temperature.
Note 7: Avoid introducing any RNases during the procedure or later handling.
Note 8: 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 16) with optional on-column DNase digestion.
Note 9: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

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.

   Note 1: Do not use more than 1x10^9 bacteria.
   Note 2: 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 lysosyme-containing TE buffer (**see note 3**). Mix by vortexing.

3. Incubate the sample at room temperature for:

   a) 3-5 min gram-negative bacteria
   b) 5-10 min gram-positive bacteria

4. Add 350 µl buffer **RL** to the sample. Mix thoroughly by vigorous vortexing.

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

5. Apply the lysate to the homogenization 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.

6. Add 250 µl ethanol (**96-100 %**) to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: A precipitate may form after addition of ethanol.

7. Apply 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.

8. Add 400 µl **Wash DN1** buffer and centrifuge for 1 minute at 11000 x g (approx. 12,000 rpm in standard laboratory centrifuges).

Note 1: 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 16) with optional on-column DNase digestion.

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

10. Add 650 µl **Wash RBW** buffer and centrifuge at 11,000 x g for 1 minute.

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

12. Add 350 µl **Wash RBW** buffer and spin down at 11,000 x g for 2 minutes.

Note 1: 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 minute.

13. Place spin-column into new receiver tube (1.5-2 ml) and add 40-60 µl RNase-free water directly onto the membrane.

Note 1: It is not necessary to close the tube at this step.

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).

YEAST RNA Purification Protocol

Note 1: This protocol is designed for isolation of total RNA (longer than 200 bases) from yeast.
Note 2: The RNA binding capacity is 100 µg per minicolumn.
Note 3: Use only freshly harvested cells.
Note 4: Prepare buffer YL 1 M sorbitol, 0.1 M EDTA. Just before use, add: 0.1 % β-mercaptoethanol, 50 U lyticase/zymolase per 1 x 10^7 cells.
Note 5: Add 10 µl β-mercaptoethanol (β-ME) 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.
Note 6: Avoid overloading the minicolumn. Overloading will significantly reduce yield and purity and may block the minicolumn.
Note 7: Avoid introducing any RNases during the procedure or later handling.
Note 8: 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 16) with optional on-column DNase digestion.
Note 9: To obtain RNA of the highest purity it is important to follow the protocol provided below carefully. During the procedure, work quickly. The yeast should be harvested at 2÷8°C. After harvesting the cells, all centrifugation steps should be performed at room temperature.
Note 10: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

1. Harvest yeast cells by centrifugation at 5000 x g for 5 min at 2÷8°C and discard the supernatant, ensuring that all liquid is completely removed.

   Note 1: Do not use more than 5 x10^7 yeast cells.

2. Resuspend cells in 1.5 ml lyticase/zymolase-containing buffer **YL** (see note 4)**.** Incubate for 30 min at 30˚C to generate spheroplasts.

   Note 1: Use only freshly harvested cells.

3. Pellet the resulting spheroplasts by centrifugation for 5 min at 1000 x g.

4. Add 350 µl buffer **RL** to lyse spheroplasts. Mix thoroughly by vigorous vortexing.

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

5. Apply the lysate 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.

6. Add 350 µl **70 %** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

   Note 1: A precipitate may form after addition of ethanol.

7. Apply the sample, including any precipitate, to the RNA binding spin-column placed in a 2 ml receiver tube. Centrifuge for for 1 minute at 11000 x g (approx. 12,000 rpm in standard laboratory centrifuges). Discard the flow-through.

8. Add 400 µl **Wash DN1** buffer and centrifuge at 11,000 x g for 1 minute.

Note 1: 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 16) with optional on-column DNase digestion.

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

10. Add 650 µl **Wash RBW** buffer and centrifuge at 11,000 x g for 1 minute.

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

12. Add 350 µl **Wash RBW** buffer and spin down at 11,000 x g for 2 minutes.

Note 1: 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 minute.

13. Place spin-column into new receiver tube (1.5-2 ml) and add 40-60 µl RNase-free water directly onto the membrane.

Note 1: It is not necessary to close the tube at this step.

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).

Appendix

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 reach in contractile proteins, connective tissue and collagen (for example: heart, muscle or skin tissue).
Note 2: The RNA binding capacity is 100 µg per minicolumn.
Note 3: Do not use more than 30 mg tissues.
Note 4: Frozen animal tissue should not be allowed to thaw during handling.
Note 5: Add 10 µl β-mercaptoethanol (β-ME) 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. Buffer RL is stable for 1 month after addition of β-ME.
Note 6: Avoid overloading the minicolumn. Overloading will significantly reduce yield and purity and may block the minicolumn.
Note 7: Avoid introducing any RNases during the procedure or later handling.
Note 8: 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 16) with optional on-column DNase digestion.
Note 9: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
Note 10: To obtain RNA 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.
Note 11: Prepare Proteinase K (20mg/ml) solution in water.

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.

   Note 1: To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.
   Note 2: Frozen tissue should not be allowed to thaw during handling.
   Note 3: 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.

   Note 1: 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.
   Note 2: We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.
   Note 3: Ensure that β-ME is added to buffer RL.

2. Add 300 µl buffer **RL** to a tissue powder. Mix thoroughly by vigorous vortexing.

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

3. Carefully transfer the sample 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.

4. Add 600 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.

   Note 1: A precipitate may form after addition of ethanol.

7. Apply 700 µl of the sample, including any precipitate, to the spin-column placed in a 2 ml receiver tube. Centrifuge for 1 minute at 11000 x g (approx. 12,000 rpm in standard laboratory centrifuges). 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 minute.

   Note 1: 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 16) with optional on-column DNase digestion.

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

11. Add 650 µl **Wash RBW** buffer and centrifuge at 11,000 x g for 1 minute.

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

13. Add 350 µl **Wash RBW** buffer and spin down at 11,000 x g for 2 minutes.

    Note 1: 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 minute.

14. Place spin-column into new receiver tube (1.5-2 ml) and add 40-60 µl RNase-free water directly onto the membrane.

    Note 1: 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 defrosting 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 U 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 $CaCl_2$ and 50 % v/v glycerol) in a concentration of 1U/µl and then add 1 U DNase I per 50 µl DNR buffer.

Note 5: DNase I is sensitive to physical denaturation. Be careful not to mix DNase vigorously.

Note 6: Use only RNase-free DNase I preparations of high quality.

Note 7: 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 minutes. Do not centrifuge.

   Note 1: 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 minute.

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 10 µl β-mercaptoethanol (β-ME) 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 2.4 µl β-ME.

1. Add 3 volumes 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 0.8 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 150 µl flow-through.

4. Apply 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. Add 400 µl **Wash DN1** buffer and centrifuge at 11,000 x g for 1 minute.

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 11,000 x g for 1 minute.

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

9. Centrifuge at 11,000 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 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.

Appendix 4:

ANIMAL TISSUE RNA Purification Protocol with PHENOL-CHLOROFORM mixture

Note 1: This protocol is designed for isolation of total RNA (longer than 200 bases) with use of Phenol-Chloroform mixture from difficult tissues reach in contractile proteins, connective tissue and collagen (for example: heart, muscle or skin tissue) or tissues deficient in RNA.
Note 2: The RNA binding capacity is 100 µg per mini column.
Note 3: 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. When using more than 120 mg of tissue, double the volume of buffer, as outlined in point 1 and 2 of the Appendix. Sample should then be applied to the column in two portions (points 4 and 6 of the Appendix). The volume of elution buffer remains unchanged.
Note 4: Frozen animal tissue should not be allowed to thaw during handling.
Note 5: 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 6: Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer LG before use. Buffer LG is stable for 1 month after addition of β-ME.
Note 7: Avoid introducing any RNases during the procedure or later handling.
Note 8: 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 16) with optional on-column DNase digestion.

Prepare a mixture of acidic phenol (pH 4.0-5.0), chloroform and isoamyl alcohol (125:24:1). Separation of nucleic acids between the phases is pH dependent. At pH 4-6 the DNA passes into the organic phase while RNA remains in the aqueous phase. RNA isolation should be carried out using acidic phenol.

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 **LG** 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 **LG** to the homogenized sample. Mix thoroughly.

   Note 1: 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 LG and to RL buffer.

2. Add 500 µl of **PHENOL-CHLOROFORM** mixture to the lysate (homogenate). Vortex for 30-60 sec.

3. Centrifuge sample for 5 min at room temperature at maximum speed to separate the aqueous and organics phases.

   Note 1: Aqueous (upper) phase contains RNA.

4. Carefully remove aqueous (upper) phase without disturbing the lower phase, and transfer it to the homogenization spin column placed in a 2 ml receiver tube. Centrifuge at maximum speed for 2 minutes.

Note 1: Be careful not to transfer remains of the interphase and the lower (organic) phase to the spin columns.
Note 2: Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.

5. Note the volume of flow-through. Add 0.5 volumes of 96-100 % ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.

Note 1: For example, if the flow-through volume is 500 µl, add 250 µl ethanol.
Note 2: A precipitate may form after addition of ethanol.

6. Apply the sample, including any precipitate, to the RNA binding spin-column placed in a 2 ml collection tube. Centrifuge for 1 minute at 11000 x g (approx. 12,000 rpm in standard laboratory centrifuges). Discard the flow-through.

7. Add 400 µl **Wash DN1** buffer and centrifuge at 11,000 x g for 1 minute.

Note 1: This step effectively eliminates the traces of remaining DNA.

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

9. Add 650 µl **Wash RBW** buffer and centrifuge at 11,000 x g for 1 minute.

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

11. Add 350 µl **Wash RBW** buffer and spin down at 11,000 x g for 2 minutes.

Note 1: 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 minute.

12. Place spin-column into new receiver tube (1.5-2 ml) and add 60-100 µl RNase-free water directly onto the membrane.

13. Centrifuge for 1 min at 11,000 x g.

14. 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).

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, 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 specifity 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 100 µg total RNA longer than 200 bases. However, it is possible to purify RNA molecules smaller than 200 bases, with gradually decreasing efficiency. Isolated RNA is ready for downstream applications without the need for ethanol precipitation.

Purified RNA is of high quality, 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 **GeneMATRI**ces, 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.

Whatever your experience with nucleic acids isolation kits may look like, most likely you will encounter a difference with **GeneMATRIX**. And, we are so much convinced, you'll love it. Enjoy.