EURx® MOLECULAR BIOLOGY PRODUCTS

Version 1.6
January 2022

GeneMATRIX Viral RNA/DNA Purification Kit

Kit for simultaneous purification of viral RNA / DNA from plasma, serum, cell-free body fluids, milk, tissues and feces.

Cat. no. E3592

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

Table of Contents

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for simultaneous isolation of viral RNA and DNA longer than 200 nt. Isolation of shorter fragments is possible, however, they are isolated with reduced efficiency. The kit allows isolation of RNA / DNA from plasma, serum or other cell-free body fluids like milk, but also blood, swabs, tissues and feces.

NOTE 2 · Maximum Sample Amount. The procedure is optimized for use with a starting volume of 200 µl. Whenever a smaller volume sample is used, bring the volume up to 200 µl with a 0.9% NaCl solution (free of RNases / DNases).

NOTE 3 · Sample Storage. After collection, plasma or serum can be stored for up to 5 hours at 2-8°C. For longer storage it is recommended to freeze samples at -20°C or -80°C in aliquots. Frozen plasma or serum samples must not be thawed more than once.

NOTE 4 · Carrier RNA. The addition of carrier RNA enhances binding of viral nucleic acids to membranes. This is particularly important in cases where the sample contains very few target molecules. In addition, the introduction of a large amount of carrier RNA reduces the chance of viral nucleic acids degradation. Dissolve Carrier RNA according to the instructions in Part I Before starting. [Carrier RNA: Poly(A) 2000-10000 nt.]

NOTE 5 · Yield of viral nucleic acids. The amount of viral nucleic acids isolated from biological samples is usually below 1 µg and is not suitable for spectrophotometric measurement. Quantitative amplification methods are recommended for determination of yields. When quantifying isolated nucleic acids remember that there will be considerably more carrier RNA in the sample than viral RNA.

NOTE 6 · Internal controls. Commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control RNA or DNA should be added together with the carrier RNA to the lysis buffer. For optimal purification efficiency, internal control molecules should be longer than 200 nucleotides.

NOTE 7 · Additional recommendations. In order to avoid cross-contamination of samples during isolation and centrifugation, particular care should be taken not to wet the rim of the column during the application of the lysate and wash buffers.

NOTE 8 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature, with the exception of Proteinase K, which should be kept at -20°C.

NOTE 9 · Maintaining Good Working Practice. All solutions should be kept tightly closed to avoid evaporation and resulting concentration changes of buffer components. To obtain high quality DNA, follow carefully the protocol provided below.

Kit content

Content	25 preps E3592-01	100 preps E3592-02	Storage/Stability
Buffer A	0.75 ml	3 ml	15-25°C
Sol V	6.5 ml	26 ml	15-25°C
Wash V1	15 ml	60 ml	15-25°C
Wash RBW	15 ml	60 ml	15-25°C
Proteinase K (20 mg/ml)	0.6 ml	2.4 ml	-20°C
RNase-free water	4.5 ml	18 ml	15-25°C
Carrier RNA	1 x 150 µg*	2 x 300 µg**	15-25°C
DNA/RNA Binding Columns	25	2 x 50	15-25°C
Protocol	1	1	

* Add 150 µl RNase free water to the tube containing 150 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl.

** Add 300 µl RNase free water to each tube containing 300 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl.

Equipment and reagents to be supplied by user

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5-2 ml tubes, a heating block capable of incubation at 60°C, vortex.
- Ethyl alcohol [96-100% v/v]. For samples <200 µl: 0.9% NaCl solution.
- BeadTubesDry Cat no. E0358 for RNA/DNA purification from feces.

Protocol

Part I Before starting

1. Dissolve Carrier RNA.

   - *Kit for 25 preps E3592-01 is supplied with one tube of 150 µg lyophilized carrier RNA, that should be dissolved in 150 µl RNase free water to obtain a solution of 1 µg/µl.*
   - *Kit for 100 preps E3592-02 is supplied with two tubes of 300 µg lyophilized carrier RNA each. Add 300 µl RNase free water to the tube to obtain a solution of 1 µg/µl.*
   - *Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C.*
   - *Use 5 µl (5 µg) of carrier RNA per one prep.*

2. Apply 25 µl of activation **Buffer A** onto the **binding 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).

   - *Addition of Buffer A onto the center of the resin enables complete wetting of membranes and maximal binding of DNA/RNA.*
   - *The membrane activation should be done before starting isolation procedure.*

3. Equilibrate samples to room temperature.

4. Add 5 µl carrier RNA suspended in RNase free water to 215 µl Sol V buffer.

   - *Carrier RNA does not dissolve in Sol V buffer. It must be dissolved in RNase free water first and then added to Sol V buffer.*
   - *The given proportion refers to one isolation. A larger volume of the mixture can be prepared according to the amount of isolation carried out.*

All centrifugation steps should be carried out at room temperature.

Part II RNA/DNA isolation

1. Pipet 20 µl **Proteinase K** into a 1.5-2 ml Eppendorf tube.

2. Add 200 µl of plasma or serum into the tube with **Proteinase K**.

   - *If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution.*

3. Add 220 µl Sol V buffer (containing carrier RNA). Close the cap and mix thoroughly by inverting the tube or vortex.

   - *Do not add Proteinase K directly to Sol V buffer.*

4. Incubate at 60°C for 15 min in a heating block.

   - *During the incubation period, mix by occasionally inverting the tube several times.*

5. To remove drops of solution from the lid, centrifuge the tubes briefly at low speed.

6. Add 250 µl of ethanol (96-100%) and mix thoroughly. Incubate the lysate with the ethanol for 1 min at room temperature.

7. Carefully transfer the sample to the **binding spin-column** placed in a 2 ml receiver tube. Close the cap and centrifuge at 8 000 x g for 1 min. Place the spin-column into the clean receiver tube and discard receiver tube containing filtrate.

8. Carefully open the column, add 500 µl Wash V1 buffer. Close the cap and centrifuge at 8 000 x g for 1 min. Place the spin-column into the clean receiver tube and discard receiver tube containing filtrate.

9. Carefully open the column, add 500 µl **Wash RBW** buffer. Close the cap and centrifuge at 8 000 x g for 1 min.

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

    - *Be careful not to contaminate the sample while removing the spin-column from the receiver tube. Check, whether the membrane of the spin column is completely dry. If not, pour off any remaining supernatant and place back spin-column into the receiver tube. Spin down for one additional min.*

11. Centrifuge at full speed for 2 min to dry the membrane completely.

12. Place spin-column in a clean microcentrifuge tube (1.5-2 ml) and add 50-100 µl **RNase-free water** directly onto the membrane.

    - *Addition of the elution buffer directly onto the center of the resin improves RNA/DNA yield. To avoid transferring traces of RNA/DNA between the spin-columns do not touch the spin-column walls with the micro-pipette.*

13. Incubate spin-column for 2 min at room temperature and centrifuge at full speed for 2 min.

14. Remove spin-column, cap the receiver tube. RNA/DNA is ready for analysis/manipulations. Isolated RNA/DNA can be stored either at 2-8°C (preferred) or at -20°C. Avoid multiple freezing and thawing.

Appendix 1: Purification of viral RNA/DNA from mucous membrane swab (including buccal, nasal, pharyngeal and vaginal swabs)

NOTE 1 • Use this protocol after „Part I Before starting" (page 5)

NOTE 2 • The swab can be taken with any commercially available swab stick.

NOTE 3 • To collect a sample, rub the inside of the cheek vigorously with a sterile swab at least 15 times.

NOTE 4 • Sample Storage. After collection, swabs can be stored for up to 5 hours at 2-8°C. For longer storage it is recommended to freeze swabs at -20°C or -80°C. Frozen swabs must not be thawed more than once.

1. Cut off the tip of the swab stick containing the sample (to allow the tube to close) and place in a 1.5-2 ml Eppedndorf tube.

2. Add 200 µl of 0.9% sodium chloride solution and 20 µl **Proteinase K** and mix.

3. Continue with step 3. Part II of the protocol „Part II RNA/DNA isolation" (page 6).

   - *DNA/RNA isolation procedure can be accelerated by simultaneous addition of 200 µl 0.9% NaCl, 220 µl SolV (with Carrier RNA), 20 µl Proteinase K to the swab and proceeding with the Part II step 4.*
   - *The swab immersed in 0.9% sodium chloride solution: transfer 200 µl of the solution to the new tube and continue with the protocol. If the obtained volume is less than 200 µl, supplement the solution with 0.9% NaCl or PBS up to 200 µl.*
   - *The swab stored in the inactivation solution (e.g. Viral Transport Medium VTM containing guanidine salts) transfer 100 µl of the solution to the new tube and add 100 µl of 0.9% NaCl or PBS.*

Appendix 2: Purification of viral RNA/DNA from animal/human feces

NOTE 1 · Use this protocol after „Part I Before starting" (page 5).

NOTE 2 · Do not exceed the weight/volume of the sample indicated in the procedure due to the efficiency reduction of the enzymatic reactions like qPCR, RT-qPCR or reverse transcription.

NOTE 3 · BeadTubeDry (Cat. no. E0358) are not supplied with the kit. To be purchased separately.

1. Weight up to 30 mg of feces (the volume of 3 rice grains) and transfer into BeadTubeDry (Cat. no. E0358).
2. Add 500 µl 0.9% NaCl or PBS and shake vigorously.
3. Spin-down briefly at 500 x g the glass beads and organic waste to obtain the clear solution.
4. Transfer 100 µl of the supernatant into the new 1.5-2 ml Eppendorf tube and add 100 µl 0.9% NaCl or PBS.
5. Add 20 µl **Proteinase K** and mix.
6. Continue with step 3. Part II of the protocol „Part II RNA/DNA isolation" (page 6).

Appendix 3: Purification of viral RNA/DNA from tissues

NOTE 1 · Use this protocol after „Part I Before starting" (page 5).

NOTE 2 · Do not exceed the amount of sample material indicated in the protocol due to the efficiency reduction of enzymatic reactions e.g. qPCR, RT-PCR and reverse transcription.

NOTE 3 · The use of Tissue Grinding Tool (cat. no E0359b) is optional. Tissue Grinding Tool is not included in this kit.

1. Weight 5-10 mg of tissue, add 400 ul 0.9 % NaCl or PBS and homogenize using mechnical homogenizer:

   - *Tissue can be grinded with liquid nitrogen to a fine powder using previously cooled mortar and pestle and take up to 10 mg of sample for further processing.*
   - *Small tissue fragment can be easily homogenized with the use of Tissue Grinding Tool Cat. no. E0359b. Insert tissue into the tube, add 400 ul 0.9 % NaCl or PBS and homogenize using the rod included in Tissue Grinding Tool.*

2. Spin down the sample shortly to collect cell debris on the bottom of the tube.

3. Transfer 200 ul of supernatant to the new 1.5-2 Eppendorf tube, add 20 ul **Proteinase K** and mix.

4. Continue with step 3. Part II of the protocol „Part II RNA/DNA isolation" (page 6).

Appendix 4: Purification of viral RNA/DNA from milk

NOTE 1 · Use this protocol after „Part I Before starting" (page 5).

1. Centrifuge 1-2 ml of milk for 3 min with the speed of 15 000 x g. Remove the supernatant carefully without disturbing the pelet (pelet can be hardly visible) and dissolve the pelet in 200 ul of 0.9 % NaCl or PBS.

2. Add 20 µl **Proteinase K** and mix.

3. Continue with step 3. Part II of the protocol „Part II RNA/DNA isolation" (page 6).

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.

Sol V

Warning

H302+H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P261 Avoid breathing vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
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.
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.
P333+P313 If skin irritation or rash occurs: get medical advice/attention.

Proteinase K

Danger

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261 Avoid breathing vapours/spray.
P304+P340 If inhaled: remove person to fresh air and keep comfortable for breathing.
P342+P311 If experiencing respiratory symptoms: call a poison center or doctor/ physician.

Wash V1

Warning

H226 Flammable liquid and vapour.
H315 Causes skin irritation.
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.
P302+P352 If on skin: wash with plenty of water.
P332+P313 If skin irritation occurs: get medical advice/attention.
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.
P403+P235 Store in a well-ventilated place. Keep cool.

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.

- ***GeneMATRIX Viral RNA/DNA Purification Kit is designed for simultaneous isolation of viral RNA and DNA. The procedure is suitable for use with plasma, serum, other cell-free body fluids and human/animal feces. Samples can be either fresh or frozen, assuming that they have not been frozen and thawed more than once.***

The procedure is optimized for use with a starting volume of 200 µl. A simple protocol based on four steps (lysis, binding, washing and elution) minimizes the time of isolation and the possibility of contamination of samples. The addition of carrier RNA supports the binding of even small amounts of viral nucleic acids. During short centrifugation RNA / DNA binds to the membrane. Unbound impurities remain in the column flow-through. Traces of contaminants remaining on the membrane are efficiently removed in two wash steps. The elution of purified RNA / DNA is carried out with RNase-free water. Effectively purified are nucleic acids with a length of over 200 nt. Isolation of shorter fragments is possible, however, they are isolated with reduced efficiency. Purified nucleic acids are free of proteins, nucleases, and other impurities and are ready for use in amplification reactions or storage at -20°C.

- **GeneMATRIX is a synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures. Novel binding and washing buffers were developed to take full advantage of GeneMATRIX capacity, yielding biologically active, high-quality nucleic acids.**

The matrix is conveniently pre-packed in ready-to-use spin-format. Due to the unique chemical composition of the matrices, in combination with optimized spin-column design, nucleic acids are isolated in outstanding quality and high purity. To speed up and simplify the isolation procedure, the key buffers are colour coded, allowing for monitoring complete mixing of mission-critical solutions, thus aiding to render the purification procedure even more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Isolated 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. One additional advantage is the high level of matrix performance reproducibility, as all components are prepared inhouse at Eurx Ltd.

EURx®
MOLECULAR
BIOLOGY
PRODUCTS

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