EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMAGNET Viral RNA/DNA Purification Kit

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

Cat. no. E3402

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

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Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for simultaneous isolation of viral RNA and DNA. 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 or PBS 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" (page 5). [Carrier RNA: Poly(A) 2 000-10 000 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 note that there will be considerably more carrier RNA in the sample than viral RNA. In the case of viral RNA/DNA purification from tissues the majority of obtained isolate consists of the genetic material of the animal/human.

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.

NOTE 7 · Additional recommendations. Avoid cross contamination of samples during isolation procedure by changing pipette tips whenever necessary.

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 and Magnetic Beads, which should be kept at 2-8°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	96 preps E3402-01	Storage/Stability
Sol V	26 ml	15-25°C
Wash V1	32 ml	15-25°C
Wash RBW	32 ml	15-25°C
Wash RBW2	32 ml	15-25°C
Proteinase K (20 mg/ml)	2.4 ml	-20°C
RNase-free water	18 ml	15-25°C
Carrier RNA *	2 x 300 µg	15-25°C
Magnetic Beads	1000 µg	2-8°C
Protocol	1	

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

Equipment and reagents to be supplied by user

- Magnetic stand E0361 for 16 tubes, E0362 for 24 tubes, E0363 for 96-well plate. To be purchased separately.
- Disposable gloves, pipettes, sterile pipette tips, sterile 1.5-2 ml tubes or 96-well plates (well volume at least 800 µl), a heating block capable of incubation at 56°C, vortex and laboratory rack for the tubes.
- Ethyl alcohol [96-100% v/v], 0.9% NaCl or PBS solution.
- BeadTubesDry Cat no. E0358 for RNA/DNA purification from feces.

Protocol

Part I Before starting

Dissolve **Carrier RNA**. Add 300 µl **RNase-free water** to the tube containing 300 µg lyophilized **Carrier RNA** 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.

1. Equilibrate samples to room temperature.

2. 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.*

Part II Samples preparation

Swab (buccal, nasal, pharyngeal and vaginal swabs):

The dry swab with biological material place in a 1.5-2 ml Eppendorf tube, add 300 µl of 0.9% NaCl or PBS and incubate for 5 min with mixing. After the incubation transfer 200 µl of the solution to the new tube.

- *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 III step 2.*

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.

Body fluids:

For the RNA/DNA isolation from blood, saliva, plasma, serum or the other cell-free body fluids collect 200 µl and transfer to 1.5-2 ml Eppendorf tube.

Milk:

Centrifuge 1 ml of milk for 3 min with 11 000 g, carefully remove the superatant without disturbing the pelet (the pelet can be hardly visible) and resuspend in 200 µl of 0.9% NaCl or PBS.

Tissues:

5-10 mg of the sample homogenize with the rotor-stator homogenizer in 400 µl of 0.9% NaCl or PBS, spin down the cellular debris (1 min 1000 g) and transfer 200 µl of the clear lysate to 1.5-2 ml Eppendorf tube.

Feces:

Weight up to 30 mg of feces (the volume of 3 rice grains) and transfer into BeadTubeDry (Cat. no. E0358). Add 500 µl 0.9% NaCl or PBS and shake vigorously. Spin-down briefly at 500 x g the glass beads and organic waste to obtain the clear solution. Transfer 100 µl of the supernatant into the new 1.5-2 ml Eppendorf tube and add 100 µl 0.9% NaCl or PBS. BeadTubeDry Cat. no. E0358 to be purchased separately.

Part III RNA/DNA isolation

1. Add 220 µl **Sol V** (containing **Carrier RNA**) and 20 µl of **Proteinase K** to the tube with biological sample.

2. Close the cap and mix thoroughly by inverting the tube or vortex. Incubate at 56°C for 15 min in a heating block.

3. Add 250 µl of ethanol (96-100%) and mix thoroughly.

   - *If working on 96-well plate format transfer the samples to the wells of the plate.*

4. Resuspend **Magnetic Beads** before removing them from the storage tube by vortexing. Add 10 µl of resuspended **Magnetic Beads** to the sample and mix by vortexing or pipeting for 1 min.

5. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

6. Remove and discard the supernatant by pipeting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 300 µl of **Wash V1** and mix by pipeting or vortexing for 10 s.

7. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

8. Remove and discard the supernatant by pipeting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 300 µl of **Wash RBW** and mix by pipeting or vortexing for 10 s.

9. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

10. Remove and discard the supernatant by pipeting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 300 µl of **Wash RBW2** and mix by pipeting or vortexing for 10 s.

11. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

12. Remove and discard the supernatant by pipeting. Leave the open tubes/plate in magnetic stand and air dry the beads for 15-20 min.

    - *Wash RBW2 contains alcohol, make sure all the solution evaporates before proceding to step 13.*

13. Add 50-100 µl **RNase-free water** to the tube/well and mix by pipeting or vortexing for 1 min.

14. Separate the magnetic beads against the side of the wells. After all the beads have been attached to the magnets transfer the supernatant containing the purified RNA/DNA to a suitable tube/plate. 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.

Safety Information

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.

Wash RBW2

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.

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.

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.

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.

- ***GeneMAGNET 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 like milk, but also with human and animal tissues and 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 to the magnetic beads, which are subsequently washed with buffers efficiently removing contaminants that could influence the efficiency of reverse transcription, PCR or qPCR. The elution of purified RNA / DNA is carried out with RNase-free water. Purified nucleic acids are free of proteins, nucleases, and other impurities and are ready for use in amplification reactions or storage at -20°C.

Nucleic acids isolation can be performed in 1.5-2 ml Eppendorf tubes or on 96-well plates with working volume 800 µl. GeneMAGNET Viral RNA/DNA Purification Kit can be used with magnetic stand for tubes or 96-well plates that can be purchased separately.

- **GeneMAGNET line is based on the use of silica paramagnetic beads (Magnetic Beads) for selective binding of RNA and DNA. The use of specially designed binding and washing buffers enables the efficient purification of highly pure nucleic acids.**

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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