IVD For *in Vitro* Diagnostic Use

CE

Magno-Virus

VIRAL RNA/DNA ISOLATION KIT

USER MANUAL

REF K-2-16/1000

NAME
Magno-Virus

INTENDED USE
The **Magno-Virus** kit is designed for the rapid, efficient magnetic preparation of highly pure viral nucleic acids (e.g. HCV, HIV, HBV, HAV, HDV, Enteroviruses, CMV) from cell free body fluids such as plasma or serum. The **Magno-Virus** test can be used in pools screening of human plasma comprised of equal aliquots of not more than 10 individual specimens (1 ml of pool composed from 10 samples).

PRINCIPLE OF ASSAY
Purification begins from the addition of Lysis buffer MV1 which is a highly concentrated solution of GITC, Binding Buffer and Magno-Beads to the tube with the clinical sample. DNA is immobilized on magnetic particle surface and contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps using Washing Buffers. The nucleic acids can be eluted in RE-buffer and are ready-for use in subsequent reactions. The prepared nucleic acids are suitable for applications like automated fluorescent RT-PCR, DNA sequencing, or any kind of enzymatic manipulation. We highly recommend the use of internal standards as well as positive and negative controls in order to monitor the purification, amplification and detection processes.

MATERIALS PROVIDED
- **Lysis Buffer MV1**, 4 x 70 ml;
- **Buffer A**, 4 x 0,6 ml;
- **Magno-Beads**, 4 x 0,9 ml;
- **Washing Buffer MV5**, 4 x 60 ml;
- **Washing Buffer MV6**, 4 x 20 ml;
- **Washing Buffer MV7***, 4 x 6,0 ml
- **RE-buffer**, 12 x 1,2 ml;

Contains reagents for 100 extractions from 500 µl or 1000 µl (up to 1000 samples in a pools screening).

MATERIALS REQUIRED BUT NOT PROVIDED
- Biological cabinet
- Vortex
- Tube racks
- Microcentrifuge tubes, 2,0 ml
- Magnetic separator for 2,0 ml tubes
- Dry block for 2,0 ml tubes
- Dry block for 5,0 ml tubes, diameter 12 mm (for RNA/DNA isolation from 1000 µl of plasma)
- Tubes 5,0 ml (for RNA/DNA isolation from 1000 µl of plasma)
- Magnetic separator for 5,0 ml tubes (for RNA/DNA isolation from 1000 µl of plasma)
- Pipettes
- Sterile, RNase-free pipette tips with filters
- Biohazard waste container
- Disposable gloves, powderless

* Washing Buffer MV7 reagent is supplied only with cargo shipments, while with air shipments only 4 empty glass tubes are supplied. Before starting tests it is necessary to add 10 ml of “Acetone for analysis” > 98% purity to each tube working in a safety biological cabinet. Note: to avoid acetone evaporation, it is necessary to close carefully the tubes.

WARNINGS AND PRECAUTIONS

-  Component Lysis Buffer MV1 contains guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/38; S: 36/37/39).
  Risk Phrases
  R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed
  R 22 Harmful if swallowed
  R 36/38 Irritating to eyes and skin
  Safety Phrases
  S 13 Keep away from food, drink and animal feedstuffs
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

SPECIMEN COLLECTION AND CONSERVATION

All kind of biological fluids or semi-fluid samples can be processed e.g. serum, urine or plasma. For successful nucleic acid purification, it is important to obtain a homogeneous, clear and non-viscous sample before loading into the corresponding isolation tube. Therefore, check all samples (especially old or frozen ones) for the presence of precipitates.
Note: Handle all specimens as if they are potentially infectious agents.

RNA/DNA isolation from plasma samples:

1. EDTA tubes may be used. Follow sample tube manufacturer's instructions.
2. Whole blood collected in EDTA should be separated into plasma and cellular components by centrifugation at 800-1600 x g for 20 min within six hours. The isolated plasma has to be transferred into a sterile polypropylene tube. Plasma may be stored at 2-8°C for 3 days. Alternatively, plasma may be stored at -18°C for up to one month or 1 year when stored at -70°C.
3. Do not freeze whole blood.
4. Specimens anti-coagulated with heparin are unsuitable for this test.
5. Thaw frozen specimens at room temperature before using. Repeated freezing and thawing leads to denaturation and precipitation of proteins, causing reduced viral titers and subsequently reduced yields of the isolated viral RNA.
6. Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma may be transported at 2-8°C or frozen.
7. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

Samples containing cells, such as cerebrospinal fluid, bone marrow, urine, should first be filtered, or centrifuged for 10 minutes at 1500 x *g* and the supernatant used.

STORAGE CONDITIONS AND PREPARATION OF WORKING SOLUTIONS

- **Magno-Virus** kit should be stored dry at +2-25°C; storage at higher temperatures should be avoided. All solutions should be stored at room temperature unless otherwise stated. **Magno-Virus** reagents can be stored for up to 1 year under the above conditions without showing any reduction in performance.
- Before use **Lysis Buffer MV1** and **Washing Buffer MV5** must be prewarmed at 60°C for a maximum of 5 min in order to redissolve salts.
- Before starting the viral RNA isolation, prepare a 70°C incubation block and preheat an aliquot of elution buffer/water.

PROTOCOL

Viral RNA/DNA isolation from 500 (1000) µl (the instructions for 1000 µl sample extraction are enclosed in brackets) cell-free biological fluids with Magno-Virus

1. Prepare required quantity of 2,0 (5,0) ml polypropylene tubes including one tube for **Negative Control of Extraction** and one tube for **RNA/DNA Positive Control of Extraction** (if provided with the amplification kit).
2. Prepare **Magnetic Mix** for 24 extractions: add into the tube with **Lysis Buffer MV1** (70 ml) **240 µl** of **Internal Control** (if provided with the amplification kit), **600 µl** of **Buffer A** and **900 µl** of **Magno-Beads.** Close the cap and carefully mix the reagents avoiding any formation of foam.
3. Add to each tube 2,0 (5,0) ml tube **1,3 (2,6) ml** of **prepared Mix**.
   *If it is necessary to test less than 24 samples add for each sample (N) in the new sterile tube **10*N µl** of **Internal Control** (if provided with the amplification kit), **20*N µl** of **Buffer A** and **30*N µl** of **Magno-Beads.** Add to each tube 2,0 (5,0) ml tube **60 µl** of **Mix** and **1,3 (2,6) ml** of **Lysis Buffer MV1.***
4. Add **500 (1000) µl** of **Samples** to the appropriate tube.
5. Prepare Controls (provided with the amplification kit) as follows:
   - add **500 µl** of **C– Negative Control** to each of the two control tubes.
   - add **100 µl** of **RNA Control C+** to the tube labeled C*pos*.
6. Mix by pipetting and incubate for 10 min at 60°C in dry block for 2,0 (5,0) ml tubes.
7. Place the 2,0 (5,0) ml tubes on the magnetic 2,0 (5,0) ml tubes separator for 6 min.
8. Carefully aspirate the supernatant with pipette leaving the magnetic particles on the wall of the tubes.
9. Take the tubes off the magnetic separator and add 700 µl of **Washing Buffer MV5.** Mix well by pipetting and vortexing. Change tips between the tubes.
   If it is used the RNA/DNA isolation from 1000 µl of plasma transfer the content of 5 ml sample tube in the new sterile 1,5 or 2,0 ml tube and perform the extraction as described below.
10. Place the tubes back on the magnetic separator for 2 min, aspirate the supernatant and discard with pipette while the tubes are situated on the magnetic separator.
11. Take the tubes off the magnetic separator and add 700 µl of **Washing Buffer MV5.** Mix well by pipetting and vortexing. Change tips between the tubes. Repeat step 10.
12. Repeat step 11 using 700 µl of **Washing Buffer MV6.**
13. Take the tubes off the magnetic separator. Add 200 µl of **Washing Buffer MV7** and mix well by pipetting. Place the tubes back on the magnetic separator for 1 min. Aspirate the supernatant and discard with pipette while the tubes are situated on the magnetic separator. Remove as much off the Washing Buffer MV7 as possible. Incubate the tubes with open caps on the magnetic separator for 10 min.
14. Add 50 (the eluation volume can be increased until 100) µl of **RE-buffer** to the tube, mix well by pipetting. Incubate at 60°C for 5 min, vortexing after 2 min.
15. Vortex briefly and place the tubes on the magnetic separator for 2 min. The supernatant contains RNA/DNA ready for amplification. Transfer the supernatant to storage tubes only while the tubes are situated on the magnetic separator.

Viral RNA/DNA is stable for up to one year when stored at –20°C or –70°C.