DBG-Spin™ Viral Nucleic Acid Kit

Description:

DBG- Spin™ Viral Nucleic Acid Kit is designed for the rapid isolation of highly pure viral nucleic acids (e.g. HCV, HIV, CMV) from biological fluids such as plasma and serum. Whole blood samples cannot be used.

The obtain nucleic acids can be directly applied for downstream applications like automized fluorescent DNA sequencing, RT-PCR, PCR, or any kind of enzymatic reactions.

Carrier RNA is included for optimal performance.

Liquid Proteinase K is included to facilitate adequate lysis of protein in the samples.

Principles of assay:

DBG- Spin™ Viral Nucleic Acid Kit is designed for DNA and RNA extraction from serum, plasma or other body fluids. Lysis is achieved by incubation of sample in a solution containing large amounts of chaotropic ions in the presence of Proteinase K. Appropriate conditions for binding of DNA to the silica membrane of the corresponding DBG- Spin™ Virus columns are performed by addition of ethanol to the lysate. Carrier RNA improved binding and recovery of low-concentrated viral nucleic acids. Potential PCR inhibitors such as salts, metabolites, and soluble macromolecular cellular compontents are removed in simple wash steps. Highly pure nucleic acids are eluted at the final step under low ionic strength conditionsin water.

Materials provided:

Lysis Buffer VL, 25 ml

Wash Buffer VW1, 30 ml

Wash Buffer VW2 (concentrate), 12 ml

Rnase-free H_2O, 13 ml

Carrier RNA (Lyophilized), 2x300 μg

Liquid Proteinase K, 600 μl

DBG- Spin Viral Nucleic acid column (plus collection tubes), 50

Collecting tubes (2 ml), 150

Collecting tubes (1.5 ml) for lysis and elution, 100

Contains reagents for 50 extractions

Material required but not provided:

Ethanol (96-100%)

Sterile, RNase free pipette tips with aerosole barrier

Disposible golves, powderless
Microcentrifuge (with rotor for 2.0 ml tubes)
Vortex mixers and Thermal heating block

Safety instructions:

- Lysis Buffer VL and Wash Buffer VW1 contain guanidine hydrochloride, which is harmful if inhaled, if it comes in contact with skin or if it is swallowed. Contact with acidic solutions releases toxic gas and combination with bleach can form highly reactive compounds. Hazards and precaution phrases: * H: 226/302/315/319/336; P: 210/260/280.
- Proteinase K sensitizer, irritant. Hazard and precaution phrases: * H:317; P:261/280.

Hazard phrases:

H 226 Flammable liquid and vapour

H 302 Harmful if swallowed

H 315 Causes skin irritation

H 317 May cause skin irritation

H 319 Causes serious eye irritation

Precaution phrases:

H 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking

H 260 In contact with water releases flammable gases which may ignite spontaneously.

H 261 In contact with water releases flammable gases

P 280 Wear protective gloves/protective clothing/eye protection/ face protection

Specimen collection and conservation:

The procedure is suitable for use with biological fluid and semi-fluid samples, such as plasma and serum. It is important to use homogeneous, clear and non-viscous samples before loading onto the DGB- Spin™ Virus columns.

Storage conditions:

DBG- Spin™ Viral Nucleic Acid Kit columns should be kept dry at room temperature (16-25 °C). All other components of the kits should be stored at room temperature upon the expiration date. After first time use, it is recommended to store Liquid Proteinases at 4 °C to -20 °C. Avoid repeated freezing and thawing of stored solutions.

Preparation of working solutions:

Before starting the protocol prepare the following:

- Add 300 µl RNase-free water to vial of carrier RNA. Store the ready solution at -20 °C.
- Add 48 ml of ethanol (96-100%) to the concentrated Wash Buffer VW2 and indicate on the bottle that ethanol was added. Store prepared buffer at room temperature (16-25 °C).

Protocol:

DNA purification with DBG- Spin™ Viral Nucleic Acid Kit

Before starting:

- Set a heating block or water bath to 70 °C.
- Preheat RNase-free H2O (for elution) to 70 °C.
- Ensure that Wash Buffer VW2 and Carrier RNA were prepared according to the instructions.

1. Pipet 5 µl Proteinase K into 1.5 ml microcentrifuge tube and add 200 µl sample (to the tune and mix moderately.

2. Add 200 µl lysis buffer VL to the samples and mix by moderate vortexing for 10-15 sec.

- If necessary, briefly centrifuge the Collection Tube (1 second at 2000 x g) to remove drop from the lid.

Add 5.6 µl Carrier RNA to the tune, mix it by vortexing or pipetting up and down.

Incubate for 3 min at room temperature.

- If necessary, briefly centrifuge the Collection Tube (1 second at 2000 x g) to remove drop from the lid.

3. Add 200 µl ethanol (96-100%) to the sample and vortex it again for 10-15 sec. Incubate for 5 min at room temperature.

- If necessary, briefly centrifuge the Collection Tube (1 second at 2000 x g) to remove drop from the lid. Do not centrifuge at a higher g-force in this step.

4. Transfer the mixture from step 3 to the DBG-Spin™ Virus Column placed in a collection tube. Centrifuge 3 min at 4000 g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (15000- 20800 g for 1 min). Discard collection tube with flow-through.

5. Place DBG-Spin™ Virus Column into a new 2 ml collectionon tube and add 400 µl wash buffer VW1 (1st wash). Centrifuge 30 second at 11000 x g. Discard collecting tube with flow-through.

6. Place DBG-Spin™ Virus Column into a new 2 ml collectionon tube and add 400 µl wash buffer VW2 (2nd wash). Centrifuge 30 second at 11000 x g. Discard collection tube with flow-through.

7. Place DBG-Spin™ Virus Column into a new 2 ml collectionon tube and add 200 µl buffer wash VW2 (Repeat 2nd wash, one more time). Centrifuge 5 min at 20000 x g (or full speed).

8. Place column in a 1.5 ml microcentrifuge tube (provided) and incubate the assembly for 5 min at 56 °C with open column lid. Add 30 µl prewarmed Rnase-free H_2O (70 °C) on to the column. Incubate at room temperature for 3 min. Centrifuge 3 min at 20000 x g to elute the nucleic acid from the column. The extracted nucleic acid is ready for your downstream applications.