DNA Spin™ Blood DNA Kit

Description:

DBG- Spin™ Blood DNA Kit is designed for the rapid isolation of highly pure genomic DNA from whole blood, serum, plasma, cultured cells, or other body fluids. It is also possible to purify viral DNA (e.g. HBV, CMV) from serum or plasma.

The kit allows purification of highly pure genomic DNA with an A260/280-ratio between 1.60 and 1.90 and a typical concentration of 40-100 ng per μl. The obtained DNA can be directly applied for down-stream applications like, PCR, Southern blotting, or any kind of enzymatic reaction.

Principles of assay:

DBG- Spin™ Blood DNA Kit is designed for genomic DNA extraction from whole blood, cultured cells, 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™ Blood columns are performed by addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Washing process remove contaminations. Highly pure genomic DNA is eluted at the final step under low ionic strength conditions in a slightly alkaline elution buffer.

The procedure is suitable for use blood samples which can be stored fresh or frozen and has been treated with citrate, heparin, or EDTA.

Materials provided:

Buffer B3, 15 ml
Wash Buffer BW, 30 ml
Wash Buffer B5 (concentrate), 12 ml
Elution Buffer BE, 13 ml
Proteinase K, 30 mg
Proteinase buffer PB, 1.8 ml
DBG- columns (plus collection tubes), 50
Collecting tubes (2 ml), 100
Contains reagents for 50 extractions

Material required but not provided:

Ethanol (96-100%)

Microcentrifuge tubes (1.5 ml)
Sterile, RNase-free pipette tips with aerosol barrier
Disposable gloves, powderless
Microcentrifuge (with rotor for 2.0 ml tubes)
Vortex mixer and Thermal heating block

Safety instructions:

- Buffer B3 and Wash Buffer BW contain guanidine hydrochloride, which is harmful if inhaled, comes into contact with skin or if swallowed. Contact with acidic solutions releases toxic gas and combination with bleach can form highly reactive compounds. Hazard and precaution phrases: * H: 226/302/319/336; P: 210/260D/264W/280sh/301+312/330.
- Proteinase K Sensitizer, irritant. Hazard and precaution phrases: * H: 315/319/334/335; P:261sh/280sh/342+311/403+233.

Hazard phrases:

H 226 Flammable liquid and vapour

H 302 Harmful if swallowed

H 315 Causes skin irritation

H 319 Causes serious eye irritation

H 335 May cause respiratory irritation

H 336 May cause drowsiness or dizziness

Precaution phrases:

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

P 260D Do not breathe vapors

P 261sh Avoid breathing dust/vapors

P 264W Wash with water thoroughly after handling

P 280sh Wear protective gloves/eye protection

P 301 + 312 IF SWALLOWED: Immediately call a POISON CENTER/doctor

P 330 Rinse mouth

P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor

P 403+233 Store in a well-ventilated place. Keep container tightly closed

Specimen collection and conservation:

The procedure is suitable for use with whole blood treated with citrate, heparin, or EDTA; buffy coat; lymphocytes. plasma; serum; and body fluids. Samples may be either fresh or frozen. Blood samples stored at room temperature or refrigerator for up to several days or weeks, will still allow DNA isolation. However, prolonged storage of blood samples under these conditions will slowly decrease DNA yield and quality due to prolonged storage of blood samples under these conditions. Blood stored frozen for years is well suited for DNA isolation. Highest yields and quality of DNA is obtained from fresh blood.

Storage conditions:

DBG- Spin™ DNA 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. During storage, especially at low temperatures, a white precipitate may form in Buffer B3. Dissolve the precipitate by incubation the bottle at 70 °C before use.

Preparation of working solutions:

Before starting the protocol prepare the following:

- Add 1.35 ml of Proteinase Buffer into the vial containing Proteinase K, to dissolve lyophilized Proteinase K. Prepared solution is stable at -20 °C for 6 months but avoid repeated freezing and thawing of stored solution. Storage of aliquots is recommended to prolong the stability of the solution.
- Add 48 ml of ethanol (96-100%) to the concentrated wash buffer B5 and indicate on the bottle that ethanol was added. Store prepared buffer at room temperature (16-25 °C) for up to one year.

Protocol:

DNA purification with DBG- Spin™ Blood DNA Kit

Before starting:

- Set a heating block or water bath to 70 °C.
- Preheat buffer wash buffer BE to 70° C.
- Ensure that wash buffer B5 and Proteinase K were prepared according to the instructions.

- 1. Pipet 25 µL Proteinase K into 1.5 ml microcentrifuge tube and add 200 µL (blood, buffy coat or body fluid sample).
    - If sample volume is less than 200 µl, add the appropriate volume of PBS.
    - If purifying DNA viruses, we recommend starting with 200 µl serum or plasma.
    - If purifying DNA from cells such as Amnion cells or cultured cells, the usage of $5x10^6$ cells in a total volume of 200 µl PBS is recommended.
- 2. Add 200 µl wash buffer B3 to the samples and mix by vigorous vortexing for 10-20 sec.
    - Note: Vigorous vortexing is important to get high yield and pure DNA.

  Incubate for 15 min at 70 °C. The lysate should become brownish during incubation with buffer B3.
    - For isolation of DNA from older or clotted blood samples, we recommend extension of Proteinase K incubation to 30 min by vortexing several times during this step.
- 3. Add 210 µl ethanol (96-100%) to the sample and vortex it again. Then briefly centrifuge the 1.5 ml microcentrifuge tube to remove droplets from the lid.
- 4. Transfer the mixture from step 3 to the DBG- Spin™ Blood column placed in a collection tube. Centrifuge 1 min at 11,000 g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (up to 15,000 g). Discard collection tube with flow-through.
- 5. Place DBG- Spin™ Blood column into a new 2 ml collection tube and add 500 µl wash buffer BW (1st wash). Centrifuge 1 min at 11,000 x g. Discard collecting tube with flow-through.
- 6. Place DBG- Spin™ Blood Column into a new 2 ml collection tube and add 600 µl wash buffer B5 (2nd wash). Centrifuge 1 min at 11,000 x g. Discard flow-through and reuse the collection tube by placing the column back to the collection tube and centrifuge 1 min at 11,000 x g.

- 7. Place column in a 1.5 ml microcentrifuge tube (not provided) and add 100 µl prewarmed elution buffer BE (70 °C). Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g. The highly pure DNA is ready for your downstream applications.