REF K-1-1/E
VER 09.02.07

Genomic Column DNA Express

USER MANUAL

NAME
Genomic Column DNA Express

INTENDED USE
Kit Genomic Column DNA Express 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, EBV) from serum or plasma.
Genomic Column DNA Express is intended as general-purpose device.
The kits allow purification of highly pure genomic DNA in less than 10 min with an A260/280-ratio between 1.60 and 1.90 and a typical concentration of 80 – 120 ng per µl.

PRINCIPLE OF ASSAY
Kit Genomic Column DNA Express is designed for genomic DNA extraction from whole blood, cultured cells, serum, plasma or other body fluids. Lysis is achieved by incubation of whole blood 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 columns are created by addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by only a single wash step. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer. The procedure, which is ideal for simultaneous processing of multiple samples, yield pure DNA ready for direct amplification in just 10 minutes. The procedure is suitable for use with fresh or frozen whole blood and blood which has been treated with citrate, heparin, or EDTA.

MATERIALS PROVIDED
- Buffer BQ1, 12,5 ml;
- Buffer BQ2(concentrate), 7 ml;
- Buffer BE, 13 ml;
- Proteinase K , 30 mg;
- Proteinase buffer, 1,8 ml;
- Genomic Column DNA Express columns (plus collection tubes), 50;
- Collecting tubes (2ml), 50;

Contains reagents for 50 tests.

MATERIALS REQUIRED BUT NOT PROVIDED
- Biological cabinet
- 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)

WARNINGS AND PRECAUTIONS
- ⚠ BQ1contain guanidine hydrochloride. Guanidine 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)*.
- ⚠ Proteinase K Sensitizer, irritant. Risk and safety phrases:* R36/37/38-42 S23-24-26-36/37

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; R42 May cause sensitisation by inhalation
Safety Phrases
S 7 Keep container tightly closed; S 13 Keep away from food, drink and animal feedstuffs; S16 Keep away from sources of ignition – No smoking; S 22 Do not breathe dust; S 24 Avoid contact with skin;
S 25 Avoid contact with the eyes; S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S 36/37/39 Wear suitable protective clothing, gloves, and eye/face protection.

- 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.

*Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12)

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 +4°C for up to several days or weeks, will still allow DNA isolation. However, DNA yield and quality will slowly decrease 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 AND PREPARATION OF WORKING SOLUTIONS

Genomic Column DNA Express columns should be stored dry at room temperature (15–25°C); storage at higher temperatures should be avoided. All solutions should be stored at room temperature unless otherwise stated.

Before starting any protocol prepare the following:

- Before first use of the kit, add 1,35 ml of Proteinase Buffer into the vial containing Proteinase K, to dissolve lyophilized proteinase K. Dissolved Proteinase is stable for up to 2 months when stored at 2–8°C. Storage at –20°C is recommended to prolong the life of Proteinase, but repeated freezing and thawing should be avoided. For this reason, storage of aliquots of Proteinase is recommended.
- Buffer BQ2 is supplied as a concentrate. Before using for the first time, add 28 ml of ethanol (96–100%). Store buffer BQ2 at room temperature (20-25°C) for up to one year.
- Upon storage, especially at low temperatures, a white precipitate may form in buffer BQ1. Dissolve such precipitates by incubation of the bottle at 70°C before use.

PROTOCOL

Genomic DNA purification with Genomic Column DNA Express

Before starting heat a water bath or heating block to 70°C. Equilibrate buffer BE to 70°C. Ensure that Buffer BQ2 and Proteinase have been prepared according to the instructions.

1. Pipet **25 µl Proteinase K** into the bottom of a 1.5 ml microcentrifuge tube and add **200 µl sample** (blood, buffy coat or body fluid sample). If the sample volume is less than 200 µl, add the appropriate volume of PBS. If purifying DNA viruses, we recommend to start with 200 µl serum or plasma.
2. Add **200 µl Lysis buffer BQ1** to the samples and mix by pulse-vortexing for 15-20 sec.
   Incubate **for 15 min at 70°C**. The lysate should become brownish during incubation with buffer BQ1. For isolation of DNA from older or clotted blood samples, we recommend extension of proteinase K incubation to 30 min and vortexing several times during this step.
3. Add **200 µl ethanol (96-100%)** to the sample and mix by vortexing for 15 sec. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
4. Apply the mixture from step 3 to the column (in a 2 ml collection tube) without wetting the rim, close the cap, and 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 to15,000 g). Discard collecting tube with flow-through.
5. Place column into a new 2 ml collecting tube and add 350 µl buffer BQ2. Centrifuge 3 min at 11,000 x g. Discard collecting tube with flow-through.
6. Place column in a 1.5ml microcentrifuge tube and add 50 µl prewarmed elution buffer BE (70°C). Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1min. Centrifuge 1 min at 11,000 x g.

SHORT PROTOCOL

Step	Description
1. Lyse	 **25 µl Proteinase K** **200 µl blood** **200 µl BQ1** **Mix** **70°C 15 min**
2. Adjust DNA	**200 µl Ethanol** **Mix**
3. Bind	 **Load sample**  **1 min 11000 x g**
4. Wash	 **350 µl BQ2**  **3 min 11000 x g**
5. Elute highly pure DNA	 **50 µl Buffer BE (70°C)** **1-2 min**  **1 min 11000 x g**