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GeneMATRIX Stool DNA Purification Kit

Kit for isolation of DNA from stool samples

Cat. no. E3575

Version 1.2

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For laboratory use only.
Not for drug, household or other uses.

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Note 1: *The kit is designed for the rapid isolation of total DNA from fresh or frozen stool samples. The isolated DNA is of high quality (contains no enzymatic inhibitors) and well suited for use in PCR and other enzymatic applications.*
Note 2: *One minicolumn enables purification of DNA from up to 200 mg of stool sample.*
Note 3: *The kit should be stored at room temperature, with the exception of PR buffer. PR buffer should be kept at 2÷8° C*
Note 4: *All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.*
Note 5: *The kit does not contain 96 % ethanol, the reagent needed during the isolation procedure.*

Protocol

1. *Apply 40 µl of activation* **Buffer ST** *onto the* **DNA binding spin-column** *(do not spin) and keep it at room temperature till transfering lysate to the spin-column.*

   Note 1: *Addition of Buffer ST onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.*
   Note 2: *The membrane activation should be done before starting isolation procedure.*

2. *Add up to 200 mg of stool sample to the* **Bead Tube**.

   Note 1: *The Bead Tube contains beads and buffer that enable dispersion of stool sample and cell lysis.*

3. *Mix vigorously by inverting till the stool sample detaches from the tube wall and suspends completely in the bead solution.*

4. *Add 60 µl* **Lyse ST** *buffer.*

   Note 1: *The components of Lyse ST buffer can form precipitate in temperature below 20°C. In this case warm the buffer up in 37°C water bath and mix well, until it gets clarified.*

5. *Vortex for 1 min.*

6. *Incubate for 5 min at 70° C.*

7. *Secure Bead Tubes horizontally using a vortex adapter tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 min.*

   Note 1: *If tubes are attached with a tape, you should be aware, that the tape may loosen. This may lead to inconsistent results or lower yields. Be sure that the tubes are tightly attached to the vortex.*
   Note 2: *Alternatively, a cell disrupter can be used. In this case processing time should be optimized.*

8. *Centrifuge the Bead Tube for 2 min at 14000 rpm and transfer 400 µl of the supernatant to the 2 ml microcentrifuge tube.*

9. *Add 400 µl* **PR** *buffer. Vortex for 5 seconds and incubate on ice for 5 min.*

   Note 1: *PR buffer precipitates non-DNA organic and inorganic material including inhibitors, cell debris, and proteins.*

10. *Centrifuge for 2 min at 14000 rpm.*

11. *Transfer 550 µl of the supernatant into a new 2 ml microcentrifuge tube.*

Note 1: *If it is impossible to transfer 550 µl of the supernatant into a new tube, transfer as much liquid as possible and adjust the volume of buffer Sol ST and 96 % ethanol proportionately in subsequent steps.*

12. *Add 650 µl* **Sol ST** *buffer.*

13. *Add 400 µl* **96 % ethanol** *and mix thoroughly by vortexing or several times inverting.*

14. *Centrifuge briefly to remove drops from the inside of the tube lid.*

15. *Transfer 600 µl of the lysate to the spin-column placed in a collection tube.*

16. *Centrifuge for 1 min at 12000 rpm.*

17. *Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.*

18. *Repeat 15-17 steps.*

19. *Transfer the remaining supernatant to the spin-column placed in a collection tube. Centrifuge for 1 min at 12000 rpm to filtrate the remains of the lysate through the resin.*

Note 1: *Continue centrifugation at 14000 rpm, if not all of the lysate passed through the column.*

20. *Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.*

21. *Add 500 µl* **Wash STX** *buffer to the spin-column and centrifuge for 1 min at 12000 rpm.*

22. *Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.*

23. *Add 500 µl* **Wash STX** *buffer to the spin-column and centrifuge for 2 min at 12000 rpm.*

24. *Place the spin-column in a new collection tube (1.5-2 ml) and add 100-200 µl of* **Elution** *buffer (10 mM Tris-HCl, pH 8.5) heated to 70°C to elute bound DNA.*

Note 1: *Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.*

Note 2: *The following elution solutions can be used:*

1. *5-10 mM Tris-HCl buffer, pH 8.0-9.0*
2. *0.5-1 x TE buffer, pH 8.0-9.0 (not recommended for DNA sequencing).*
3. *Other special application buffers can be used, if their pH and salt concentration is similar to that of 5-10 mM Tris-HCl, pH 8.0-9.0.*

25. *Incubate the spin-column/collection tube assembly for 2 min at room temperature.*

26. *Centrifuge the spin-column for 30 seconds at 12000 rpm.*

27. *Discard spin-column, cap the collection tube. DNA is ready for analysis/manipulation. It can be stored either at 2÷8° C or at -20°C.*

GeneMATRIX *is synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures. Novel binding and washing buffers are developed to take full advantage of* **GeneMATRIX** *capacity, yielding biologically active, high-quality nucleic acids. Matrix is conveniently pre-packed in ready-to-use spin-format. Unique chemical composition of the matrixes along with optimized construction of spin-columns improve the quality of final DNA or RNA preparation. To speed up and simplify isolation procedure, the key buffers are colour coded, which allows monitoring of complete solution mixing and makes purification procedure more reproducible.*

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Such DNA or RNA can be directly used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybrydization among others. Additional advantage is reproducibility of matrix performance, as component preparation is carried at Eurx Ltd.

GeneMATRIX Stool DNA Purification Kit *is designed for the rapid isolation of highly pure, total DNA from fresh or frozen stool samples. Purified DNA is free of contaminants, such as: enzymatic inhibitors, proteins, lipids, dyes, detergents, buffers, salts, divalent cations, etc.*

Stool sample is added to a bead beating tube containing beads and lysis solution. The principal is to lyse human (or animal) cells and the microorganisms in the stool sample by a combination of heat, detergent and mechanical force against the beads. Specialized solution is added to precipitate inhibitors that strongly inhibit downstream applications. Optimized buffer and ethanol provide selective conditions for DNA binding to the DNA binding spin-columns. Contaminants remaining on the resin are efficiently removed in two wash steps. High-quality cellular DNA is then eluted in low salt buffer, e.g.: Tris-HCl, TE. Isolated DNA is ready for downstream applications without the need for the ethanol precipitation.