EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Stool DNA Purification Kit

Kit for isolation of DNA from stool samples

Cat. no. E3575

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Content	50 preps E3575-01	100 preps E3575-02	Storage/Stability
Buffer ST	1.8 ml	3.6 ml	15-25°C
Lyse ST	3.6 ml	7.2 ml	15-25°C
PR	24 ml	48 ml	2-8°C
Sol ST	39 ml	78 ml	15-25°C
Wash STX	60 ml	120 ml	15-25°C
Elution	6 ml	12 ml	15-25°C
DNA Binding Columns	6 ml	12 ml	15-25°C
Bead Tube	50	2 x 50	15-25°C
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Introductory Notes

NOTE 1 · Kit Specification. 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 · Maximum Sample Portion. One minicolumn enables purification of DNA from up to 200 mg of stool sample. The maximum column binding capacity for DNA is 25 µg. The maximum volume of the column reservoir is 650 µl.

NOTE 3 · Kit Compounds Storage. The kit should be stored at room temperature, with the exception of PR buffer. PR buffer should be kept at 4°C.

NOTE 4 · Maintaining Good Working Practice. All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes. To obtain high quality DNA, stick carefully to the protocol provided below.

Equipment and reagents to be supplied by the experimenter.

- Ethanol [96–100% v/v], microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml collection tubes. Equipment for stool sample disruption and homogenization: a flat-bed vortex pad or cell disrupter (FastPrep, Precellys, Disruptor Genie, etc.). Heating block capable of incubation at 70°C.

Protocol

1. Apply 30 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 (for best results at least 10 min).

   - *Addition of Buffer ST onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.*

   - *The membrane activation should be done before starting isolation procedure.*

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

   - *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. Vortex for 1 min.

   - *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. Incubate for 5 min at 70°C.

6. Secure **Bead Tube** 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.

   - *Alternatively, a cell disrupter (FastPrep, Precellys, Disruptor Genie, etc.) can be used, what enables to achieve higher yield. In this case processing time should be optimized.*

   - *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.*

7. Centrifuge the **Bead Tube** for 2 min at 12 000 x g and transfer 400 µl of the supernatant to the 2 ml microcentrifuge tube.

8. Add 400 µl **PR** buffer. Vortex for 5 sec and incubate on ice for 5 min.

   - *PR buffer precipitates non-DNA organic and inorganic material including humic substances, cell debris, and proteins.*

9. Centrifuge for 1 min at 12 000 x g.

10. Transfer 550 µl of the supernatant to a new 2 ml microcentrifuge tube.

    - *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.*

11. Add 650 µl **Sol ST** buffer.

12. Add 400 µl of ethanol (96–100%) and mix thoroughly by vortexing or several times inverting.

13. Transfer 600 µl of the lysate to the **DNA binding spin-column** and centrifuge at 11 000 x g for 30 sec. Remove the spin-column, pour off supernatant and place back into the receiver tube.

14. Repeat step 13.

15. Transfer the remaining mixture to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

16. Add 500 µl **Wash STX** buffer to the spin-column and centrifuge for 1 min at 11 000 x g.

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

18. Add 500 µl **Wash STX** buffer to the spin-column and centrifuge for 1 min at 11 000 x g.

19. Spin down at 11 000 x g for 1 min to remove traces of the **Wash STX** buffer.

20. Place the spin-column in a new collection tube (1.5–2 ml) and add 50–100 µl of **Elution** buffer to elute bound DNA.

    - *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.*

    - *In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.*

    - *The following elution solutions can be used:*
      - *5–10 mM Tris-HCl buffer, pH 8.0–9.0.*
      - *0.5–1 x TE buffer, pH 8.0–9.0 (not recommended for DNA sequencing).*
      - *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.*

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

22. Centrifuge the spin-column for 1 min at 11 000 x g.

23. 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.

Safety Information

Buffer ST

Danger

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+**P330**+**P331** If swallowed: Rinse mouth. Do not induce vomiting.
P303+**P361**+**P353** If on skin (or hair): take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a poison center/doctor.
P405 Store locked up.

Sol ST

Warning

H302+**H332** Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P261 Avoid breathing vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+**P312** If swallowed: call a poison center/ doctor if you feel unwell.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333+**P313** If skin irritation or rash occurs: get medical advice/attention.
P337+**P313** If eye irritation persists: get medical advice/ attention.
EUH208 Contains ethylenediammonium dichloride. May produce an allergic reaction.

Wash STX

Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+**P235** Store in a well-ventilated place. Keep cool.
P337+**P313** If eye irritation persists: get medical advice/ attention.

GeneMATRIX is synonymous for a family of synthetic, new generation, nucleic acid binding membranes.

The GeneMATRIX membrane family has gained fame for two striking features: First, for their extraordinary high binding capacity, allowing to isolate nucleic acids with optimal yield. Second, for their remarkably high specificity. Even compounds of pronounced chemical similarity such as DNA, RNA and polysaccharides are easily differentiated amongst each other by the selectivity of these highly optimized matrices. This feature allows to isolate highly pure nucleic acids, that remain to work reliable even after being subjected to extended storage periods (years). Or, directly upon isolation, when used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybridization, etc ...

We take great care to fine-tune all components and variables of the nucleic acid purification system towards each other – a multi-parameter optimization.

All matrices ship conveniently pre-packed in a ready-to-use spin-column format. Spin columns are specifically constructed for precise adjustment of liquid flow-through rate to optimal values. Novel binding and washing buffers are developed to take full advantage of GeneMATRIX unique capacity, resulting in isolation of biologically active, high-quality nucleic acids. And, last not least, a lot of time and efforts went into development of the various GeneMATRIces, thus providing a platform of unique chemical composition.

High and continuous reproducibility of matrix performance is always warranted, since component preparation as well as stringent quality control is entirely performed in-house at EURx Ltd.

Whatever your experience with nucleic acids isolation kits may look like, most likely you will encounter a difference with GeneMATRIX. And, we are so much convinced, you'll love it. Enjoy.

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.

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