EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Environmental DNA & RNA Purification Kit

Fraction, isolate and purify genomic DNA and total RNA.
For stool, soil and environmental samples.

Cat. no. E3572

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Table of Contents

Content	25 preps E3572-01	100 preps E3572-02	Storage/Stability
Buffer EN	0.75 ml	3 ml	15-25°C
Extraction EN	23 ml	90 ml	15-25°C
AFR01	0.1 ml	0.4 ml	15-25°C
DRP	6 ml	24 ml	15-25°C
DLT	10 ml	39 ml	15-25°C
BL *	3 ml	12 ml	–20°C
Proteinase K (20 mg/ml)	0.6 ml	2.4 ml	–20°C
Wash RBW	36 ml	144 ml	15-25°C
RNase-free water	6 ml	24 ml	15-25°C
Elution	3 ml	12 ml	15-25°C
DNA Binding Columns	25	2 x 50	2-8°C
RNA Binding Columns	25	2 x 50	2-8°C
Bead Tube Dry	25	2 x 50	15-25°C
Protocol	1	1	

* Contains lysozyme (20 mg/ml).

Introductory Notes

NOTE 1 · This kit is designed for isolation of genomic DNA and total RNA (together with small RNA molecules) from a single biological sample. It allows to fraction and purify DNA and RNA from lysis-resistant sample material, such as stool, soil and environmental samples.Typical contaminants are removed efficiently, especially humic substances.

NOTE 2 · The kit gives particulary good results in the isolation of DNA/RNA from bacteria present in environmental samples.

NOTE 3 · DNA binding capacity is 25 µg per spin-column. Loading more than 25 µg DNA may lead to DNA contamination of the RNA eluate.

NOTE 4 · The RNA binding capacity is 120 µg per spin-column.

NOTE 5 · Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.

NOTE 6 · Once the kit is unpacked, store components at room temperature, with the exception of BL buffer (with lysozyme) and Proteinase K, which should be kept at -20°C. Store DNA Binding Columns and RNA Binding Columns in 2-8°C. All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

NOTE 7 · Add 50 µl β-mercaptoethanol (β-ME) per 10 ml Extraction-EN buffer before use. Extraction-EN buffer is stable for 1 month after addition of β-ME.

NOTE 8 · To prevent excessive foaming of the mixture during homogenization add 50 µl AFR01 reagent per 10 ml Extraction-EN buffer before use. Extraction-EN buffer with AFR01 reagent added can be stored for 3 months.

NOTE 9 · Measurement of nucleic acid concentrations: In general, the concentration of DNA and RNA isolated from soil/stool/environmental samples is not reliably measurable by spectrophotometric methods, since the A_{260}/A_{280} ratio for isolated nucleic acids from such samples is likely not between 1.8 and 2.0. Use fluorescent measurement for assessing DNA and RNA concentrations adequately (for an example protocol see page 9).

NOTE 10 · Elution buffer is a low salt solution, that contains no metal ion chealators (e.g. EDTA) that can inhibit subsequent enzymatic reactions. Elution buffer composition is suitable for downstream applications such as digestion with restriction enzymes, phosphorylation, ligation, Sanger sequencing, NGS etc. It is also possible to elute the DNA with Tris-HCl, water or TE.

The kit contains glass beads for efficient mechanical cell disintegration. Due to the broad biological diversity in composition of environmental samples, this kit uses a universal approach towards homogenization and non-selective cell lysis by combining two independent lysis methods: (1) mechanical trituration using glass beads of different diameters in an detergent-rich environment, (2) enzymatic action of lysozyme, Proteinase K and optional enzymes of the experimenter's choice (if required). Special composition of extraction buffer allows to separate DNA/RNA from humic acids and protect nucleic acids from degradation. Moreover, extraction with organic solutions (no phenol) removes humic and polyphenolic inhibitors of enzymatic reactions. Consequently, this method allows to achieve satisfactory efficiency of simultaneous isolation of genomic DNA

and RNA from the same sample without contamination of humic substances. Purified nucleic acids are applicable for reliable enzyme digestion, hybridization, reverse transcription, and PCR amplification. Main optimization focus of the kit is to provide best compatibility of purified nucleic acids with further enzymatic treatment in downstream applications.

Equipment and reagents to be supplied by the experimenter

- Equipment for sample disruption and homogenization: a flat-bed vortex pad or cell disrupter (FastPrep, Precellys, Disruptor Genie, etc.). Cell disruptors maximize DNA/RNA yields, but require careful optimization of shaking time (generally, a reduction compared to the time specified in the protocol).
- Microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5–2 ml tubes, a heating block capable of incubation at 37–55°C.
- Ethanol [96–100% v/v], chloroform, isopropanol, β-mercaptoethanol (14.3 M, β-ME).

Sample Material and Sample Sizes

Sample Material	**Maximum Sample Size**
Stool samples	up to 200 mg stool sample
Soil samples	up to 250 mg wet soil, sediment or slurry sample material or up to 100 mg dry sample material
Biofilms	up to $10E^9$ cells
Filters from filtrated water samples	up to 1 cm diameter
Cell pellets of pure cultures from lysis-resistant microorganisms with deviant cell wall composition (e.g. gram-positive bacteria, archaea, ...)	up to $10E^9$ cells

Table 1: Maximum recommended sample sizes for sample material from various sources, where mechanical cell disruption is conducted by vortexing. Rotor-stator homogenization is more efficient, as compared to sample homogenization by vortexing. Thus sample sizes for rotor-stator homogenization should be reduced. Homogenization efficiency for rotor-stator homogenizers is device-dependent - determine optimal sample sizes empirically. Maximum required sample sizes for rotor-stator homogenization may be as low as $1/10^{th}$ of the sample amount required for homogenization by vortexing.

Highest purity and best yield is obtained, if sample sizes are kept small. Extraction efficiency of the kit is optimal with rather small sample sizes, well below the upper borders given in the table above.

Protocol

Part I Disruption, Mechanical Lysis, Phase separation

1. Apply 25 µl of activation **Buffer EN** onto the **DNA binding spin-column** (do not spin) and keep it at room temperature until transfer of lysate to spin-column.

   - *Addition of Buffer EN 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. The minimum activation time is 5-15 min.*

2. Add 650 µl **Extraction-EN** buffer to the **BeadTubeDry**.

   - *Ensure that AFR01 reagent is added to buffer Extraction EN (see page 3 , note 8).*

   - *Cell pellets or biofilms, should first be suspended in 650 µl Extraction-EN buffer and then added to the BeadTubeDry.*

   - *Use up to 1cm^2 of filter. Filter from filtrated water sample should be washed with 650 µl Extraction-EN buffer and then buffer should be added to the BeadTubeDry. Also, filter can be cut into a small pieces and added to the BeadTubeDry with Extraction-EN buffer.*

   - *Work swiftly, avoid clump formation.*

   - *Buffer Extraction-EN serves the following purposes: (I) Cell lysis, (II) immediate protection of DNA and RNA from degradation, when released through cell lysis, (III) protection DNA/RNA from reaction with humic substances, (IV) neutralizing humic substances by coagulation.*

3. Add up to 200 mg of stool or up to 250 mg of soil sample to the **BeadTubeDry** with Extraction-EN buffer.

   - *If slurry, wet sediments or liquid samples are added, sample volume should not exceed 200 µl.*

4. Mix vigorously by inverting until the sample material detaches from the tube wall and suspends completely in the solution.

5. Incubate for 5 min at 55°C.

6. Secure **BeadTubeDry** horizontally to a vortex by using a vortex adapter or a tube holder. Vortex at maximum speed for 5 min.

   - *Alternatively, a cell disruptor (FastPrep, Precellys, Disruptor Genie, etc.) may be used. Maximum DNA/RNA yields are achieved by using a cell disruptor rather than by vortexing. But, for preventing excessive DNA/RNA fragmentation, it is required to optimize the shaking time (generally, a time reduction, as compared to the time specified above for vortexing, depending on the specific type of cell disruptor in use).*

- *To reduce the foam volume generated during vortexing, spin down the sample shortly (11 000 x g for 30 s).*

7. Add 325 µl buffer **DLT** to the **BeadTubeDry**. Mix by several-fold inverting.

   - *Buffer DLT provides optimal working conditions for enzymes attacking and digesting cell walls, while protecting DNA and RNA from degradation and ensuring that nucleic acids are not lost during centrifugation steps.*

   - *Although not required, it is possible to optionally add additional enzymes for specific digestion of cell walls in case certain specific target organisms require being detected within the sample. Such enzymes are e.g. zymolase and lyticase (yeasts) or lysostaphin (Streptococcus).*

8. Next add 100 µl **BL** buffer to the **BeadTubeDry**. Mix by several-fold inverting or vortex 3 sec. Incubate **BeadTubeDry** at room temperature for 15 min.

   - *Optimal incubation temperature is 37°C.*

   - *If bacteria are not main target skip this step and continue with point 10 of the Protocol.*

9. Again secure **BeadTubeDry** horizontally to a vortex by using a vortex adapter or a tube holder. Vortex at maximum speed for 2 min.

10. After vortexing add 20 µl **Proteinase K** to the **BeadTubeDry**. Mix by several-fold inverting the tube.

11. Incubate for 30 min at 55°C.

    - *During the incubation period, mix by occasionally inverting the tube several times.*

12. Again secure **BeadTubeDry** horizontally to a vortex by using a vortex adapter or a tube holder. Vortex at maximum speed for 5 min.

13. Centrifuge the **BeadTubeDry** for 2 min at 12 000 x g and transfer as much as possible of the supernatant to the 2 ml microcentrifuge tube. Note the volume of supernatant.

    - *Volume of supernatant which can be taken depends on the nature of the sample. Usually it is about 600 µl.*

14. Add 0.5 volumes of chloroform. Cover the sample tightly and vortex for 2 min.

15. Centrifuge sample at 12 000 x g for 3 min at room temperature.

    - *Centrifugation separates the mixture into 3 phases: organic phase (bottom, usually darker), an interphase and upper aqueous phase (containing DNA and RNA). The upper aqueous phase is ~50% of the total volume.*

16. Remove upper, aqueous phase very carefully, without disturbing the interphase and transfer into a new 2 ml microcentrifuge tube. Note the volume.

Part II DNA/RNA Precipitation

1. Add 0.5 volumes of 100% isopropanol to the aqueous phase and mix by several-fold inverting.
2. Incubate samples for 15 min at room temperature.
3. Centrifuge sample at 12 000 x g for 15 min at room temperature and carefully decant the supernatant.
   - *Carefully pipette to remove the remaining supernatant.*
   - *Drying a pellet is not necessary.*
4. Suspend the pellet in 100 µl of **RNase-free water**. Mix thoroughly by pipetting.
   - *Pellet is usually invisible.*
5. Add 200 µl **DRP** buffer to the suspended pellet. Mix thoroughly by pipetting.
6. Apply the mixture to the **DNA binding spin-column** placed in a 2 ml collection tube. Centrifuge at 11 000 x g for 1 minute. Use the flow-through for RNA purification. Continue immediately with part III, point 1 of the protocol (RNA isolation, page 7).
7. Store the DNA binding spin column at room temperature 15-25°C or at 2-8°C for later DNA purification (part IV of the protocol).

Part III RNA Purification

1. To the flow-through from the step 6 of part II of the protocol add 300 µl of ethanol (96-100% [v/v]). Mix thoroughly. Do not centrifuge.
2. Apply the mixture to the **RNA binding spin-column** placed in a 2 ml collection tube. Centrifuge for 1 min at 11 000 x g. Remove the spin-column, pour off supernatant and place back into the receiver tube.
3. Add 600 µl of **Wash RBW** buffer and spin down at 11 000 x g for 1 minute.
4. Remove the spin-column, pour off supernatant and place back into the receiver tube.
5. Centrifuge at 11 000 x g for additional 1 minute to remove any residual wash buffer.
6. Place spin-column into new receiver tube (1.5–2 ml) and add 40–60 µl **RNase-free water** directly onto the membrane.
7. Centrifuge for 2 min at 11 000 x g .
8. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Store the samples at -20°C or below.

Part IV Genomic DNA Purification

1. Add 600 µl of **Wash RBW** buffer to the DNA binding spin column (from the last step of part II of the protocol) and centrifuge at 11 000 x g for 1 minute.
2. Remove the spin-column, pour off supernatant and place back into the receiver tube.
3. Centrifuge at 11 000 x g for additional 1 minute to remove any residual wash buffer.
4. Place spin-column into new receiver tube (1.5–2 ml) and add 40–60 µl of **Elution** buffer directly onto the membrane to elute bound DNA.
5. Incubate spin-column/receiver tube assembly for 2 minutes at room temperature.
6. Centrifuge for 2 min at 11 000 x g .
7. Remove spin-column, cap the receiver tube. Genomic DNA is ready for analysis/ manipulations. It can be stored either at 2-8°C (preferred) or at -20°C (avoid multiple freezing and defrosting of DNA).

Appendix: Fluorescent determination of DNA and RNA Concentration

1. Prepare working solutions for dyes (e.g. 1:2000 of RiboGreen or PicoGreen in 1x TE buffer, pH 7.0).

   - *Dyes are not included with the kit and have to be purchased separately from third parties.*
   - *It is possible to use other DNA- and RNA-specific dyes.*
   - *Dye names are trademarks owned by third parties.*

2. As a reference, prepare a dilution series of DNA and RNA in 1:10 dilution steps. Prepare a stock of 2 µg/ml and verify concentration by spectrophotometric measurement:

   dsDNA: 2 µg/ml dsDNAcorresponds to A_{260} = 0,04
   RNA: 2 µg/ml RNA corresponds to A_{260} = 0,05

   Dilute reference solutions in 1: 10 steps. DNA: e.g. calf thymus DNA starting from 1 µg/ml DNA; RNA: e.g. E.coli 16 + 23S rRNA starting from 100 ng/µl RNA.

   - *For the given dye concentrations, linear ranges of calibration lines are typically 0.5 – 20 ng for DNA (PicoGreen) and 0.5 – 30 ng for RNA (RiboGreen) per 200 µl sample volume.*

3. Mix 100 µl of nucleic acid solution with 100 µl of dye working solution.

4. Transfer 200 µl solution to a microtiter plate suitable for fluorescent meaurement and measure in a microplate reader at appropriate wavelengths for excitation and emission. (e.g. RiboGreen and PicoGreen: ~480 nm excitation, ~520 nm emission). Work swiftly and protect dyes from light as far as possible to avoid bleaching of fluorescent dyes.

Safety Information

Buffer EN

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.

Extraction EN

Warning

H319 Causes serious eye irritation.
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.
P337+**P313** If eye irritation persists: Get medical advice/ attention.

Proteinase K

Danger

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261 Avoid breathing vapours/spray.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
P342+**P311** If experiencing respiratory symptoms: call a poison center or doctor/ physician.

DRP

Warning

H302+**H332** Harmful if swallowed or if inhaled.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
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.
EUH032 Contact with acids liberates very toxic gas.

Wash RBW

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 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 Environmental DNA & RNA Isolation Kit is designed for rapid fractioning, isolation and purification of genomic DNA and total RNA starting from one single biological sample. Efficient lysis and cell disruption is warranted due to non-selective mechanical lysis supported by enzymatic treatment of the sample. This gives particulary good results when the bacteria are major target of the resercher.

Protocol outline: Sample material is mechanically disrupted, homogenized and lysed in the presence of special extraction buffer, which protect nucleic acids before degradation and reaction with humic substances. Bacterial or yeast cells are lysed in the presence of special cell wall disintegrating buffer aided by lysozyme (bacteria). The pretreated cell wall is then effectively destroyed by the mechanical friction of glass beads with optimally adjusted diameters. Further, Proteinase K digests cellular proteins, including stripping-off DNA of all bound proteins, among them nucleases. During the next steps, chloroform is added, and the homogenate is allowed to separate into a clear upper aqueous layer, an interphase, and a lower organic layer. Nucleic acids can be precipitated from the aqueous layer with isopropanol and separeted into DNA and RNA fraction using minicolumns and wash buffers. This kit is ideal for researchers who are interested in parallel studies of metagenomes and metatranscriptomes of a single sample.

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