

GeneMATRIX Environmental DNA & RNA Purification Kit

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

Cat. no. E3572

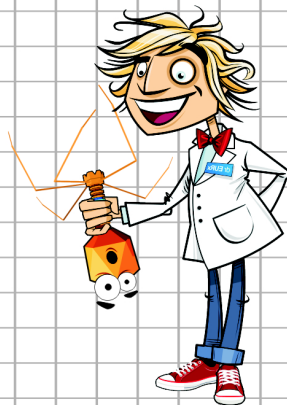
Version 1.0

October, 2016

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

NUCLEIC ACIDS ISOLATION KITS GUIDE

SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL		ISOLATION OF DNA															ISOLATION OF RNA												
		E3600	E3540	E3580	E3510	E3545	E3560	E3555	E3525	E3520	E3595	E3535	E3500	E3565	E3515	E3570	E3575	E3530	E3550	E3551	E3596	E3594	E3597	E3598	E3599	E3593			
		MICELLULUA DNA	AGAROSE - OUT DNA	BACTERIAL & YEAST GENOMIC DNA	BIO - TRACE DNA	BASIC DNA	BONE DNA	CELL CULTURE DNA	FOOD EXTRACT DNA	PCR / DNA CLEAN-UP	PLANT & FUNGI DNA	AGROBACTERIUM PLASMID DNA	PLASMIID MINIPREP DNA	QUICK BLOOD DNA	SHORT DNA CLEAN-UP	SOIL DNA	STOOL DNA	SWAB-EXTRACT DNA	TISSUE DNA	TISSUE & BACTERIAL DNA	HUMAN BLOOD RNA	UNIVERSAL BLOOD RNA	UNIVERSAL DNA/RNA /PROTEIN	UNIVERSAL RNA	UNIVERSAL RNA /miRNA	FFPE RNA Purification Kit			
		50 150	50 150	50 150	25 100	50 150	25 50	50 150	25 100	50 150	50 150	50 150	50 150	50 150	25 100	50 100	50 100	25 100	50 150	50 150	25	25	25 100	25 100	25 100	25 100			
DNA	GENOMIC	BACTERIA		●															●										
		YEAST		●																									
		CELL CULTURE						●											●	●									
		PLANT AND FUNGI										●																	
		PLANT RICH IN POLYSACCHARIDES ²										●																	
		BLOOD												●															
		SOIL															●												
		STOOL																●											
		SWAB																	●										
		SOLID TISSUES																		●	●								
		LIQUID TISSUES																		●	●								
		FFPE TISSUE SECTIONS																		●	●								
		RODENT TAILS																		●	●								
		HAIR																		●	●								
		INSECTS																		●	●								
	URINE																		●	●									
	BONE																												
	BIOLOGICAL TRACES				●																								
	FOOD								●																				
	PLASMID	BACTERIA																											
YEAST				●																									
ISOLATION FROM AGAROSE GELS			●			●																							
PURIFICATION OF PCR PRODUCTS / DNA AFTER ENZYMATIC REACTIONS		●				●			●																				
DNA/RNA/PROTEIN FROM THE SAME BIOLOGICAL SAMPLE	ANIMAL TISSUE																						●						
	PLANT TISSUE																						●						
	BACTERIA																						●						
	YEAST																						●						
	CELL CULTURE																						●						
RNA	TOTAL RNA LONGER THAN 200 BASES	ANIMAL TISSUE																						●		●			
		PLANT TISSUE																							●		●		
		BACTERIA																							●		●		
		YEAST																							●		●		
		CELL CULTURE																							●		●		
	HUMAN BLOOD	FRESH																			●	●							
		FROZEN ¹																			●	●							
	ANIMAL BLOOD	FRESH																			●	●							
		FROZEN ¹																			●	●							
	miRNA OR TOTAL RNA	ANIMAL TISSUE																									●		
FFPE TISSUE SECTIONS																											●		
PLANT TISSUE																											●		
CELL CULTURE																											●		
PURIFICATION OF RNA AFTER ENZYMATIC REACTIONS																									●	●			
ON-COLUMN DNase DIGESTION																									●	●			



All kits contain buffers WASH in ready to use form

1. Frozen with the addition of Lyse Blood buffer (included in kit).
2. Additionally required lyse CT buffer (E0324)

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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 a 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 1/10th 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.

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 particularly 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. 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: 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 10).

Protocol

Part I Disruption, Mechanical Lysis, Phase separation.

1. Apply 25 μ l 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.

Note 1: Addition of Buffer EN 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 650 μ l **Extraction-EN** buffer to the **Dry Bead Tube**.

Note 1: Cell pellets or biofilms, should first be suspended in 650 μ l Extraction-EN buffer and then added to the Dry Bead Tube.

Note 2: Small filters (up to 1 cm diameter) from filtrated water samples should be washed with 650 μ l Extraction-EN buffer and then buffer should be added to the Dry Bead Tube. Also, filter can be cut into a small pieces and added to the Bead Tubes with Extraction-EN buffer. For larger filters (up to 4.7 cm diameter) use large scale environmental kit.

Note 3: Work swiftly, avoid clump formation.

Note 4: 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 **Bead Tube** with **Extraction-EN** buffer.

Note 1: If slurry, wet sediments or liquid samples are added, sample volume should not exceed 200 μ l.

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 **Bead Tube** horizontally to a vortex by using a vortex adapter or a tube holder. Vortex at maximum speed for 5 min.

Note 1: 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).

7. Add 325 μ l buffer **DLT** to the **Bead Tube**. Mix by several-fold inverting.

Note 1: 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.

Note 2: 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 μ l **BL** buffer to the **Bead Tube**. Mix by several-fold inverting or vortex 3 sec. Incubate **Bead Tube** at room temperature for 15 min.

Note 1: Optimal incubation temperature is 37°C.

Note 2: If bacteria are not main target skip this step and continue with point 10 of the Protocol.

9. Again secure **Bead Tube** 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 μ l **Proteinase K** to the **Bead Tube**. Mix by several-fold inverting the tube.

11. Incubate for 30 min at 55°C.

Note 1: During the incubation period, mix by occasionally inverting the tube several times.

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

13. Centrifuge the **Bead Tube** 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.

Note 1: Volume of supernatant which can be taken depends on the nature of the sample. Usually it is about 600 μ l.

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.

Note 1: 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.

Note 1: Carefully pipette to remove the remaining supernatant.

Note 2: Drying a pellet is not necessary.

4. Suspend the pellet in 100 μ l of **RNase-free water**. Mix thoroughly by pipetting.

Note 1: Pellet is usually invisible.

5. Add 200 μ l **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 11000 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 8).
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 μ l 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 11000 x g. Remove the spin-column, pour off supernatant and place back into the receiver tube.
3. Add 600 μ l of **Wash RBW** buffer and spin down at 11000 x g for 1 minute.
4. Remove the spin-column, pour off supernatant and place back into the receiver tube.
5. Centrifuge at 11000 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 μ l **RNase-free water** directly onto the membrane.
7. Centrifuge for 2 min at 11000 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 μ l of **Wash RBW** buffer to the DNA binding spin column (from the last step of part II of the protocol) and centrifuge at 11000 x g for 1 minute.
2. Remove the spin-column, pour off supernatant and place back into the receiver tube.
3. Centrifuge at 11000 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 μ 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 11000 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).

Note 1: Dyes are not included with the kit and have to be purchased separately from third parties.

Note 2: It is possible to use other DNA- and RNA-specific dyes.

Note 3: 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 dsDNA corresponds 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.

Note 1: 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 measurement 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.

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 particularly good results when the bacteria are major target of the researcher.

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 separated 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.

GeneMATRIX is a 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 matrices 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, hybridization among others.