User Manual

VIOGENE
www.viogene.com

Ver.22A

Viogene eDNA Water Extraction System

Viogene eDNA Water is designed for the isolation of eDNA from 20 ml of environmental water samples such as river, stream, creek, lake, pond, lagoon, bay, sea or ocean. The eDNA Water recovers small to large eDNA fragments. A simple one step gravity filter-column protocol without arduous vacuum or pressure filter steps. DNA yield strongly depends on the individual sample type and processed water volume. Total DNA yields of a few **ng up to 2 µg** have been observed from 20 ml of environmental water. Isolated DNA is suitable for diverse downstream applications such as PCR, qPCR and metabarcoding.

Product Contents

Cat. No	EDW1001
Preps	30
LYS Buffer	40 mL
EDB Buffer	80 mL
WS Buffer	30 mL
Elution Buffer	5 mL
Proteinase K	20mg x 4
EDW Filter Column	30
50ml tube	30
Mini Plus Column	60
Protocol	1

Reagents, consumables, and equipment to be supplied by user

Reagents and consumables

- All procedures expect 55 ℃ incubation should be processed at room temperature (20 - 25 ℃)
- Add 120 ml of 98 -100 % ethanol to the WS Buffer bottle when first opened.
- Eppendrof microcentrifuge tubes (1.5 mL) tube for elution
- Disposable pipet tips (aerosol barrier pipet tips are recommended)
- DNA decontamination solution, e.g. bleach or Virkon Aquatic for decontamination of reusable materials and surfaces (e.g. water collection device, lab bench, pincers).

Equipment

- Water collection device (e.g. bottle, bucket, can, canister, beaker)
- Manual pipettors
- Personal protection equipment (lab coat, gloves, goggles)
- Centrifuge for 50 mL tubes with a swing-out rotor capable of reaching 1,500 x g for use of extraction protocol
- Centrifuge for microcentrifuge tubes (1.5 mL or 2 mL) capable of reaching 10,000 RPM

For Soils DNA extraction

Measure a certain amount of soil and soak into certain amount of clean water for overnight, after overnight soaking of the soil sample, ster up the soil and water mixture for two minutes, let the sample sit for five minutes to settle the soil into the bottom of the container, then using the upper part of the water as the environmental water for eDNA extraction by follow the protocol.

Protocol

1. Take one 50 mL tube plus EDW filter column, unscrew the cap of the tube and hold up the EDW filter column.

2. Hold the EDW filter column at the edge, and fill up the EDW filter column with eWater, avoid finger touching the eWater to prevent cross-contamination.

3. Screw cap back the eWater filled EDW filter column to the 50 ml tube and label the tube.

4. Let the eWater gravity flow through the EDW filter column, about 20 ml of eWater will flow through.

5. Discard the collected eWater of the through eWater, put back the EDW filter column into the empty 50 ml tube.

6. Centrifuge 3 minutes of the 50 ml tube plus EDW filter column by 1,500 x g, and discard the flow through water in the bottom of the 50 ml tube..

7. Add 1 ml of LYS buffer into one tube of dry 20 mg proteinase K, and dissolve the proteinase K by vortexing, and store the dissolved proteinase K in 4℃.

8. Use a new 1.5 ml tube, pipette 1 ml LYS buffer and 100 ul of dissolved proteinase K in the LYS buffer into the 1 ml LYS buffer/tube, then pipette the 1.1 ml proteinase K plus LYS buffer in to centrifuged EDW filter column inside the 50 ml tube, close the cap and then incubate the column/tube at 55℃ incubator.

9. Incubate the LYS plus proteinase K buffer/centrifuged EDW filter column/tube at 55℃ for 2 hours or overnight.

10. After incubation, centrifuge 3 minutes of EDW filter column plus tube by 1,500 x g.

11. Around 1 ml of centrifuged proteinase K treated eWater is in the bottom of the 50 ml tube, then discards the EDW filter column.

12. Add 2 ml of EDB buffer into the 1 ml proteinase K treated eWater elute.

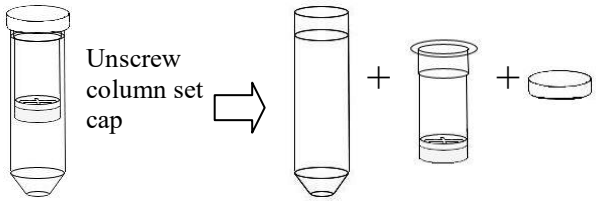
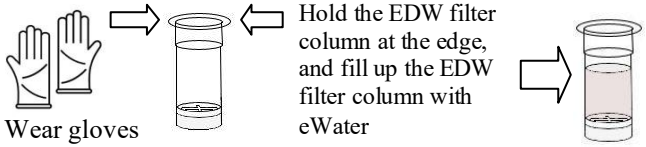
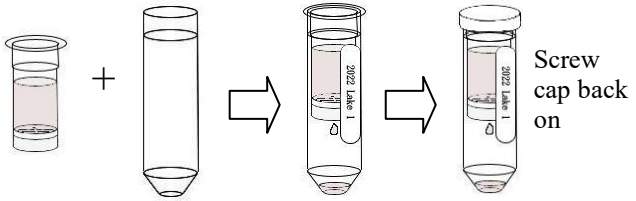
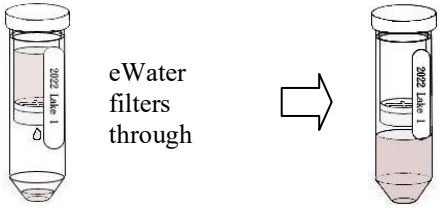
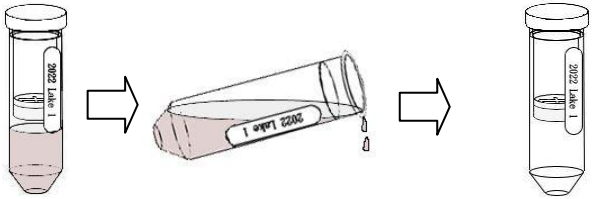
13. Take two mini plus columns set (with collection tubes), load 0.75 ml GP plus eWater elute mixture into each of the column, then centrifuge 1 minutes at 10,000 RPM to pass the sample mixture into the column, discard the flow through, repeat the loading and centrifugation again to complete passing the sample mixture into the columns.

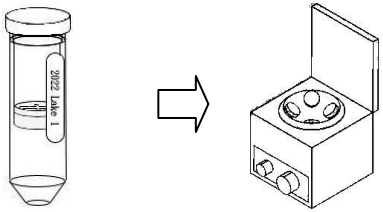
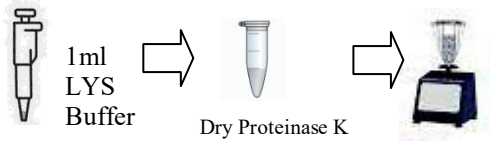
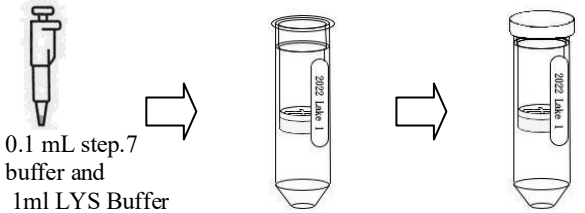
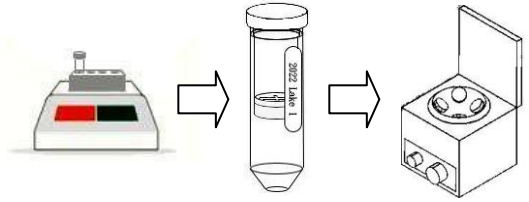
14. Wash twice of the columns with 0.7 ml of ethanol added WS buffer by centrifugation 1 minute at 10,000 RPM.

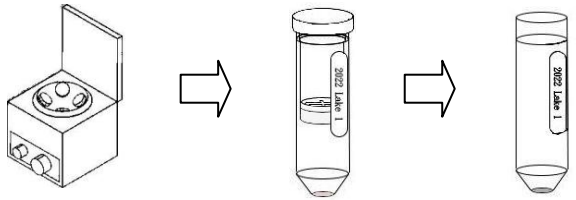
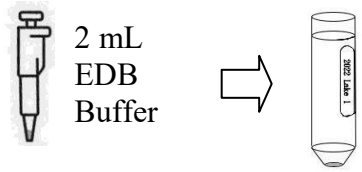
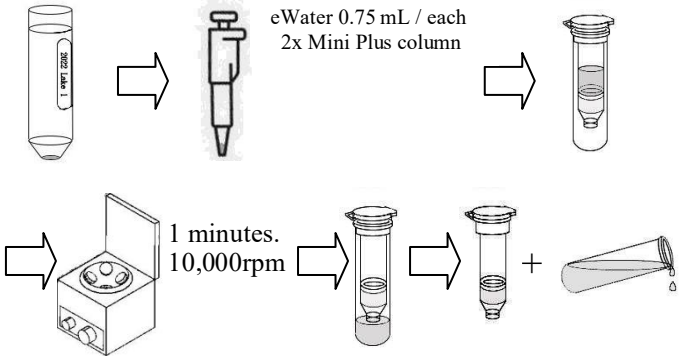
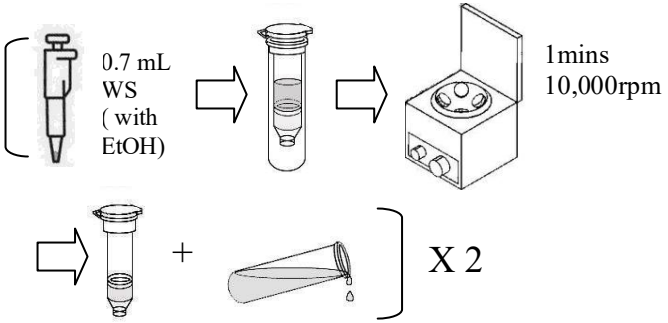
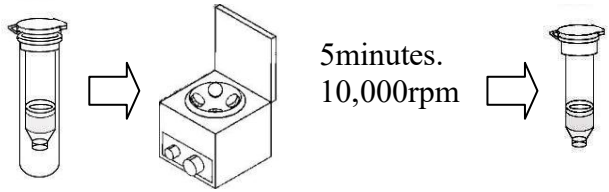
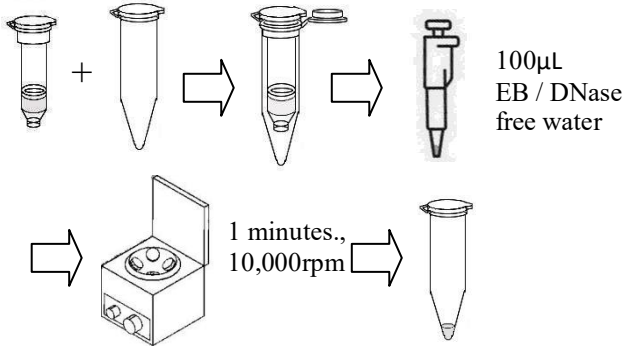
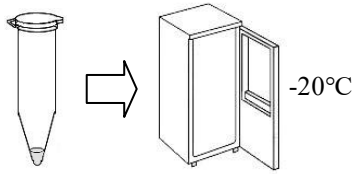
15. Dry the columns by another 5 minute centrifugation at 10,000 RPM.

16. Take two new 1.5 ml eppendrof and insert the dried columns into, then add 100 ul of Elution Buffer or DNAse free water.

17. Store the samples in -20℃ or for downstream application.

1	Column set : 50 mL tube + EDW filter column	
2	EDW filter column + eWater	
3	EDW filter column + 50 mL tube + label	
4	Filter eWater: Set column upright and let eWater filter out	
5	Discard eWater	

6	Centrifuge for 3 minutes at 1500 x g	
7	1 mL LYS Buffer + dry Protenase K let dissolve	
8	Add 0.1 mL step 7 buffer and 1mL LYS Buffer to Step 6 column	
9	Incubate the column at 55°C for 2 hrs or overnight	
10	Centrifuge the column for 3 minutes at 1500 x g	

11	Discards the EDW filter column	
12	2ml EDB Buffer to the column	
13	Load 0.75 mL / each into 2x Mini Plus columns, centrifuge 1minutes / 10,000rpm, discard the flow through	
14	Wash 0.7 mL WS(with EtOH) twice, centrifuge for 1 minute at 10,000 rpm before discarding the WS wash	
15	Dry columns, centrifuge for 5 minutes at 10,000rpm	
16	Insert each dry columns into 1.5 mL eppendrof tube add 100 µL Elution Buffer / DNase free water	
17	Storage -20°C	

Product description

The basic principle: Viogene eDNA Water - filtration column method

Viogene eDNA Water is a kit intended for the isolation of eDNA from around 20 milliliter water samples collected from natural freshwater and marine environments. The focus of the kit is the isolation of DNA released from diverse organisms into the water. Organisms targeted are for example populations of fish, amphibians, reptiles, crustaceans, mammals, birds, plants and other organisms that came in contact with the sampled water.

eDNA can be described as any DNA released from organisms into the environment. With regard to water samples, eDNA can be derived from e.g. epithelial cells, excretions, decaying organisms or pieces of tissue, reproductive cells (e.g. sperms, spawn, pollen). Most eDNA is associated with particles, such as cells, organelles, or bound to grains of inorganic or organic material. For this reason, eDNA can be obtained from water samples by filtration.
After filtration of an adequate water volume, DNA from the water is retained on the filter of the column by gravity flow. DNA is released from the filter and with a special dissolving buffer and proteinase. Subsequently, DNA is absorbed to a particular minicolumn matrix, washed and finally eluted with a low salt elution buffer.

Product specification

- Viogene eDNA Water is designed for the isolation of eDNA from up to 20 ml of environmental water samples such as river, stream, creek, lake, pond, lagoon, bay, sea or ocean.
- Viogene eDNA Water recovers small to large eDNA fragments.
- A filtration protocol is supported by the kit.
- DNA yield strongly depends on the individual sample type and processed water volume. Total DNA yields of a few ng up to 2 µg have been observed from 20 ml of environmental water
- Isolated DNA is suitable for diverse downstream applications such as PCR, qPCR and metabarcoding.

Handling of sample material - Stability of eDNA in the water sample

With collection / removal of a water sample from its natural environment, changes of eDNA amount and distribution in the sample will start. Sample storage can significantly influence the stability of the eDNA. For best results, the time from sample collection to onset of eDNA isolation should not exceed 24 hours. Ideally, water samples should be filtrated in the field at the collection site. If direct filtration is not an option, water samples can be cooled to 0 °C – 4 °C for several hours before eDNA extraction.

Stability of DNA on the filter

eDNA can be stabilized on a filter by briefly passing ethanol (2 mL) over the filter in order to dry the filter. The ethanol-wet filter can be stored for several days in a closed tube at ambient temperature avoiding a spoilage of the sample/filter.

Elution procedures

A volume of 100 µL is recommended for eDNA elution from the Viogene eDNA column. A smaller volume for elution is not recommended.

Size, yield and quality of eDNA obtained from water samples

DNA fragments from approximately 100 bp to >5,000 bp have been observed.Yield of total DNA can be in the range of a few ng up to 2 µg per 20 ml water sample. Average fragment length and concentration of the isolated eDNA depend on the water sample being processed.

Stability of isolated DNA

Due to the low DNA content of typical water samples, the resulting low total amount of isolated DNA, fragmentation, and the absence of DNase inhibitors, the eluates should be

kept on ice for short term storage and frozen at -20°C or below for long term storage for optimal results.

Storage conditions and preparation of working solutions

Attention: EDB Buffer contains chaotropic salt! Wear gloves and goggles!

Caution: EDB buffer contains chaotropic salt which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- Dry Proteinase K can be stored at room temperature (18–25 °C) and is stable for at least one year. However, store Liquid Proteinase K at 4 °C or -20 °C after first use.
- All other kit components should be stored at room temperature (18–25 °C) and are stable for at least one year.
- Storage / transport at lower temperatures may cause precipitation of salt in the buffer. If any precipitate is visible, heat the solution to 50°C for 30 min while mixing it, let the solution cool down to room temperature.

Safety instructions

The following components of the Viogene eDNA kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g

Component

Hazard contents

DANGER

- EDB buffer

  Guanidine Hydrochloride <CAS number 50-01-1>

  Potassium acetic acid <CAS number 127-08-2>

  Acetic acid <CAS number 64-19-7>

- Wash Buffer WS

  Tris Hydrochloride <CAS number 1185-53-1>

  Tris Base <CAS number 77-86-1>

  EDTA <CAS number 6381-92-6>

- LYS Buffer

  Tris Hydrochloride <CAS number 1185-53-1>

  Tris Base <CAS number 77-86-1>

  EDTA <CAS number 6381-92-6>

  Sodium dodecyl sulfate < CAS number 151-21-3>

Highly flammable liquid and vapor.

Causes severe skin burns and eye damage.

May cause an allergic skin reaction.

DANGER

The symbol shown on labels refers to further safety information in this section.

Hazard phrases

Keep away from heat/sparks/open flames/hot surfaces. No smoking.

Keep the container tightly closed.

Do not breathe dust/vapors.

Wash with water thoroughly after handling.

Avoid release to the environment.

Wear protective gloves/eye protection.

IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.

IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Immediately call a POISON CENTER/doctor.

Rinse mouth.

For further information please see Material Safety Data Sheets

Storage / transport of filters (optional)

If storage / transport of the filter is required after filtration of water, add 2 mL of ethanol (p.a. quality) and let it pass through the filter. The ethanol displaces residual water from the filter and thus conserves the eDNA on the filter. Place the ethanol-wet filter into a suitable tube, e.g. a 2 mL reaction tube (e.g. Eppendorf Biopur grade).

After storage/transport of the filter, remove the filter from the tube and briefly let residual ethanol evaporate, before starting the eDNA release step.

Troubleshooting

Possible cause and suggestions

- DNA contamination during sample collection or processing: Make sure to work in an environment which minimizes risk of DNA contamination: close doors and windows; prevent uncontrolled air draft; wear clean personal protection equipment; change gloves frequently; ideally work in a controlled positive pressure lab.
- Contaminated reusable materials (e.g. water collection container): Make sure to only use reusable materials which has been decontaminated.
- Contaminated working environment: Decontaminate your working environment (e.g. lab bench surfaces) before starting the procedure.
- Run a no-sample extraction control in order to obtain information, which DNA does not originate from your sample.

The Viogene eDNA Water kit is produced under controlled and monitored conditions with high hygienic standards. Nonetheless, it cannot be guaranteed that there is no contaminating DNA at all times of whatever source due to the omnipresence of DNA and the increasing sensitivity of DNA detection and assignment. Therefore, a no-sample extraction control (mock preparation) is recommended.

Product use restriction / warranty

Viogene eDNA Water products are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original Viogene product leaflets. Viogene products are intended for GENERAL LABORATORY USE ONLY! Viogene products are suited for QUALIFIED PERSONNEL ONLY! Viogene products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! Viogene products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. Viogene does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

It cannot be guaranteed that the Viogene eDNA Water kits are free of any detectable DNA at all times. However, the products are produced under controlled and monitored conditions with high hygienic standards to keep the risk of DNA contamination of whatever source as low as reasonably possible.