INSTRUCTIONS FOR USE

UNEX Buffer

INTENDED USE

UNEX Buffer is a guanidium isothiocyanate-based lysis buffer developed by the Centers for Disease Control and Prevention (CDC) and used for Universal Nucleic Acid Extraction (UNEX) of DNA and RNA from viruses, bacteria, and parasites from various sample types. This product is not intended to diagnose, treat, cure or prevent disease.

MATERIALS REQUIRED BUT NOT PROVIDED

100% and 70% Molecular-grade ethanol
10 mM Tris-EDTA pH 8.0
Spin columns [e.g. HiBind® mini column (Omega BioTek) and Econospin mini column (Epoch Life Science)]
Collection tubes
Sterile microcentrifuge tubes

OPTIONAL MATERIALS

Lytic enzymes (e.g. Proteinase K)
Beads (e.g., 0.2 mm and 0.5 mm zirconium oxide or glass beads)

INSTRUCTIONS FOR USE

General Protocol

The CDC has developed protocols for extraction of RNA/DNA from different specimens. Each protocol below represents example methods for extracting nucleic acids from bacteria, viruses or parasites. End-user developed modifications may be required depending on sample size and type.

Important Points Before Starting

- Do not mix bleach with the UNEX buffer or its waste. It is recommended to add UNEX buffer or its waste to a bottle of sodium hydroxide (0.3M) to prevent the formation of toxic gases.
- Always place a spin column into a collection tube or microcentrifuge tube before centrifuging.
- The elution volume may vary depending on the size of column selected. A larger volume might be required to allow complete rehydration of column membrane. This will result in more a dilute nucleic acid concentration

Protocol for Nucleic Acid Extraction from Bacterial and Viral Samples

A. Lysis

1. Add 150 µl of clarified sample to a microcentrifuge tube.
2. Add 150 µl of UNEX lysis buffer and mix.
3. Incubate for 10 minutes at room temperature.

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UNEX BUFFER

B. Binding

4. Add 300 µl of 100% ethanol and mix.
5. Transfer all of the mixture to a spin column in a collection tube and close the cap.
6. Centrifuge the spin column at 13,000 x g for 1 minute. Discard filtrate in a container with 0.3 M Sodium hydroxide.

C. Washing

7. Add 500 µl of 70% molecular grade ethanol to the spin column.
8. Centrifuge the spin column at 13,000 x g for 1 minute. Discard filtrate.
9. Repeat steps 9 to wash the spin column again.
10. Centrifuge the spin column at 13,000 x g for 1 minute to remove residual ethanol.

E. Eluting

11. Transfer the spin column in a new microcentrifuge tube. Discard the filtrate and the collection tube.
12. Add 50-100 µl of 10 mM Tris-1 mM EDTA (pH 8.0) buffer or nuclease-free water to the center of the spin column.
13. Centrifuge the spin column at 13,000 x g for 1 minute. Discard the spin column.
14. Transfer the filtrate containing purified nucleic acids to a new microcentrifuge tube. Store at 2°C-8°C for immediate use or at -70°C or colder for later use.

Protocol for Nucleic Acid Extraction from Parasites in Water Samples

A. Lysis

1. Using a microcentrifuge tube cap, add a “capful” (approximately 200 mg) of the zirconium oxide or glass beads into each bead beating tube.
2. To a bead beating tube:
   a. Add 350 µl of UNEX nucleic acid extraction buffer.
   b. Add 350 µl of the water sample.
   c. Add 35 µl of Proteinase K to bead beating tube.
3. Vortex the bead beating tube for 15 seconds to mix.
4. Incubate for 15 minutes at room temperature (for Proteinase K activity).
5. Place the bead beating tube securely in bead beating instrument and set bead beating instrument to appropriate speed and time.
6. Centrifuge the bead beating tube at 10,000 x g for 1 minute.

B. Binding

7. Transfer supernatant (avoiding pellet) from each bead beating tube to a spin column in a collection tube.
8. Centrifuge the spin column at 10,000 x g for 1 minute. Discard the filtrate in a container with 0.3 M Sodium hydroxide.

C. Washing

9. Add 500 µl of 100% molecular grade ethanol to the spin column.
10. Centrifuge the spin column at 10,000 x g for 1 minute. Discard the filtrate.
11. Add 500 µl of 70% ethanol to the spin column.
12. Centrifuge the spin column at 10,000 x g for 1 minute. Discard the filtrate.
13. Centrifuge the spin column again at 10,000 x g for 1 minute to remove residual ethanol.

D. Eluting

14. Transfer the spin column in a new microcentrifuge tube. Discard the filtrate and the collection tube.
15. Add 50-100 µl of 10 mM Tris- 1 mM EDTA (pH 8.0) buffer or nuclease-free water to the center of the spin column.
16. Centrifuge the spin column at 10,000 x g for 1 minute. Discard the spin column.
17. Transfer the filtrate containing purified nucleic acids to a new microcentrifuge tube. Store at 2°C-8°C for immediate use or at -70°C or colder for later use.

PRECAUTIONS AND LIMITATIONS

- Do not mix bleach with the UNEX buffer or its waste. It is recommended to add UNEX buffer or its waste to a bottle of sodium hydroxide (0.3M) to prevent the formation of toxic gases.
- For research use only.
- Not intended for human, animal or pet consumption.
- Refer to the SDS for more detailed information. The SDS can be located on our website at www.microbiologics.com or by contacting Technical Support at 320.229.7045 or U.S. Toll Free 866.286.6691.
- Only trained laboratory personnel should use this product.

STORAGE AND EXPIRATION

Store the UNEX Buffer at 15˚C-30˚C and protect from light. Stored as directed, UNEX Buffer will retain, until the last day of the month of the expiration date stated on the product label, its specifications and performance within the stated limits.

The UNEX Buffer should not be used if:

- Stored improperly
- There is evidence of excessive exposure to heat or moisture
- The expiration date has passed

FORMULA COMPONENTS

UNEX Buffer is an aqueous solution containing the following components: CAS 593-84-0, CAS 6381-92-6, CAS 77-86-1, CAS 7647-14-5, CAS127-09-3, CAS 151-21-3CAS 9005-64-5, CAS 6892-68-8, CAS 7757-83-7 and CAS 26763-19-9.

KEY OF SYMBOLS

Batch Code (Lot)

Catalog Number


Warning


Manufacturer


Temperature Limitation


Use By

PRODUCT WARRANTY

- These products are warranted to meet the specifications and performance printed and illustrated in product inserts, instructions, and supportive literature.
- The warranty, expressed or implied, is limited when:
  - The procedures employed in the laboratory are contrary to printed and illustrated directions and instructions
  - The products are employed for applications other than the intended use cited in product inserts, instructions, and supportive literature

WEBSITE

Visit our website, **www.microbiologics.com**, for current technical information and product availability.

REFERENCES

1) Hill, V.R., Jothikumar, N., Vinjé, J., Cromeans, T.L. 2010. Sample Preparation Methods for Molecular Techniques for Drinking Water. Project Report #3108. Water Research Foundation.

2) J.M. Shields, J. Joo, R. Kim, H.R. Murphy. 2013. Assessment of three commercial DNA extraction kits and a laboratory-developed method for detecting Cryptosporidium and Cyclospora in raspberry wash, basil wash and pesto. J. Microbiol. Meth., 92:51–58.

3) Hill VR, Mull B , Jothikumar N, Ferdinand K and Vinje J. 2010. Detection of GI and GII Noroviruses in Ground Water Using Ultrafiltration and TaqMan Real-time RT-PCR. Food and Env Virology 2(4):218-224.

4) Bonnie J. Mull, Jothikumar Narayanan, and Vincent R. Hill, 2013. Improved Method for the Detection and Quantification of Naegleria fowleri in Water and Sediment Using Immunomagnetic Separation and Real-Time PCR. J. Parasitol. Res., Article ID 608367, 8 pages.

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