AquaStool™

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MOLECULAR BIOTECHNOLOGY

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General Information

Application: DNA/RNA extraction from fecal & other specimens. For *in vitro* research use only.

Size: The kit is sufficient for the preparation of:

7001MT: 1 ml for 6 extractions on mouse fecal pellets
7030MT: 30 ml for 200 extractions on mouse fecal pellets

Kit Contents: The AquaStool Kit includes the following items:

7001MT: 1 ml AquaStool Solution, Instruction Manual
7030MT: 30 ml AquaStool Solution, Instruction Manual

Note: Order AquaRemove (# 1208MT) separately for human fecal DNA/RNA purification. Dilute 8 ml AquaRemove with 8 ml isopropanol before use.

Description

Stool is an accessible and noninvasive source of biospecimen. Fecal DNA/RNA originated from the host, intestinal bacteria, viruses, fungi, or parasites, and incompletely digested foods are molecular fingerprints of the host and its health. AquaStool is a multifunctional aqueous reagent for fecal sample stabilization, DNA and RNA extraction and PCR inhibitor removal. It may be used to extract fecal DNA for non-invasive genotyping of transgenic animals. AquaStool may be used to preserve and extract DNA/RNA from human stools for host and gut microbiome research. AquaStool may also be used to extract DNA from tree tissues, which are particularly challenging with existing DNA extraction methods.

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Product Warning: Contains guanidine thiocyanate, is harmful if swallowed and causes irritation to skin, eyes and respiratory tract. Do not mix with Bleach.

A. Mouse fecal DNA extraction and genotyping

This protocol uses 150 µl of AquaStool to extract DNA from a mouse fecal pellet. Each extraction is sufficient for a hundred PCR reactions. Use 300 µl AquaStool for DNA extraction from a rat fecal pellet.

1. **Fecal sample collection:** Transfer a mouse to a clean cage (set up 5-10 cages at a time) floored with a clean diaper or paper towel. Fecal pellets usually appear in a few minutes. Label each microfuge tube with corresponding mouse ID. Scoop up a fecal pellet with the microfuge tube or its lid (*Important: Double check the mouse ID and tube ID to avoid mislabeling the fecal pellet*). After the collection, place the mice back to their original cages. Replace the diaper in each collection cage and start another round of collection. The fecal pellets may be air-dried in an open tube on a 37 °C dry heat block for 24 hrs. The air-dried fecal pellets may be stored long-term at room temperature for future DNA extraction and genotype verification.

2. **Fecal DNA extraction:** Pre-load each microfuge tube with ~25 mg of white sand (Sigma # 274739, white, 50+70 mesh) and 150 µl of AquaStool. Add one mouse fecal pellet into each tube. Vortex (use a pipette tip to break up the pellet if needed) at top speed for 1-2 min to fully homogenize the fecal material. Incubate at 22-65 °C for 15-30 min, Centrifuge at 12,000 xg for 5 min to pellet the debris. (If a multi-tube beadbeater such as BioSpec Mini-Beadbeater-24 is available for homogenization, 24 fecal samples can be processed simultaneously)

3. **Pellet the fecal DNA:** Transfer the supernatant (~90 µl, avoid taking up the fecal debris) to a new 0.5-µl microfuge tube. Add 0.8 vol (~72 µl) of isopropanol and vortex to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA/RNA pellet air-dry for 5-10 min. Add 100 µl of TE buffer or deionized water to the DNA pellet, pipette or vortex vigorously to suspend the DNA. Centrifuge at 12,000 xg for 10 min to pellet any insoluble, which contains fecal PCR inhibitors, and then transfer the clear DNA/RNA solution to a new tube and store at -20 °C.

4. **PCR genotyping:** Set up a 25 µl PCR reaction containing 1 µl of fecal DNA (It is important to centrifuge the frozen DNA solution again before its use in a PCR reaction to pellet any insoluble that may have developed during the storage). Use appropriate primer pairs in the same PCR reaction to amplify the transgene and a control host gene with 45-65 thermal cycles.

B. Human fecal DNA/RNA extraction and analysis

Note: An additional PCR inhibitor removing reagent, AquaRemove, is recommended for human fecal DNA/RNA purification. Please order AquaRemove (#1208MT) separately. Before using AquaRemove, add an equal volume (8 ml) of 100% isopropanol to the AquaRemove solution (8 ml). Shake vigorously to mix well. Keep the cap of the bottle airtight to prevent isopropanol from evaporating out during storage.

1) Stool Collection and Preservation

This protocol describes the use of 10 ml of AquaStool solution to collect one gram of human feces. However, using 0.5 ml of AquaStool solution to collect 50 mg of feces may be sufficient for most applications.

1. Transfer a level spoonful (~1 gram) of fresh stool into a 15-ml stool collection tube (SARSTEDT # 80.734.311) pre-loaded with 10 ml of AquaStool solution.

2. Stir and smash the stool with the spoon to facilitate the contact of the specimen with AquaStool solution. Securely close the screw cap and shake the content vigorously a few times.

3. Transfer the stool specimen to the laboratory. (*AquaStool preserved sample is stable at room temperature and may be shipped at ambient temperatures. However, if the intention is to extract RNA, the samples should be shipped in wet or dry ice*.)

2) Stool Specimen Storage

To streamline subsequent fecal DNA and RNA extractions, upon receiving the stool specimens in the laboratory, each sample is preferably divided into 0.5-ml aliquots, each containing ~50 mg of fecal materials, in 1.5-ml microfuge tubes for storage as described below.

1. Vortex the stool specimen in the stool collection tube vigorously at top speed to fully homogenize the specimen.

2. Add ~100 mg of white sand (Sigma # 274739, white, 50+70 mesh) into 1.5 ml microfuge tubes. Sand assists homogenization of the fecal matter and is required for bacterial cell lysis and bacterial DNA/RNA extraction. It is convenient to use the cap of a 0.2 ml PCR tube to scoop and add the sand into the 1.5 ml tubes. One capful of sand is ~90-100 mg.

3. Transfer 0.5 ml of the homogenized stool specimen into the 1.5 ml microfuge tubes pre-loaded with sand (cut off the tip of a 1-ml blue pipette tip and use it to transfer the sample to avoid the tip being clogged by the fecal debris).

4. Label the tubes and store the samples at -80 °C for long-term storage.

3) Fecal DNA/RNA extraction by bead beating (This method will not shear the DNA):

1. Extract the DNA/RNA: Retrieve 0.5 ml stool specimen in a 1.5 ml microfuge tube containing ~100 mg of sand from storage. Vortex the tube upside down at top speed for a few minutes (use a multichannel bead beater, if available). Add 250 µl of isopropanol-diluted (1:1 dilution) AquaRemove to the crude lysate. Vortex and incubate at 22 °C for 5-10 min. Centrifuge at 14,000 xg for 5 min to pellet the fecal debris.

2. Precipitate the DNA/RNA: Transfer 0.6 ml cleared lysate to a 1.5 ml tube and add 0.8 volumes (0.48 ml) isopropanol. Vortex to mix. Centrifuge at 12,000 xg for 5 min to pellet the DNA/RNA (*Proteins remain in the isopropanol supernatant and may be recovered by precipitation in 4 vol of acetone or by dialysis*). Decant to discard the supernatant. Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA/RNA pellet air-dry for 5-10 min.

3. Solubilize the DNA/RNA: Add 0.4 ml deionized water to the pellet, pipet and vortex to disperse the pellet. Incubate at 22 °C for 15 min and then centrifuge at 12,000 xg for 5-10 min to pellet any insoluble. Transfer the clear DNA/RNA solution to a new tube and store at -20 or -80 °C (Note: Centrifuge the frozen DNA solution to remove any insoluble before its use in a PCR reaction.).
The expected DNA/RNA concentration is ~200 ng/ml. The total DNA/RNA yield from 0.5 ml of AquaStool-stabilized stool specimen (~50 mg of feces) should be about 80-100 µg.

4) Fecal DNA/RNA extraction by sonication

(This method will shear the DNA, which may be used directly for next-generation sequencing. The RNA may be converted to cDNA for qPCR analysis or next-generation sequencing.):

1. Extract the DNA/RNA: Retrieve 0.5 ml stool specimen in a 1.5 ml microfuge tube containing ~100 mg of sand from storage. Place a lid clamp to prevent the lid from popping up during sonication. Put the tube on a foam floater and immerse the tube in the water bath of a bath sonicator (Branson Ultrasonic Cleaner 2510, 40 kHz, Danbury, CT). (*Note: It is critical to position the tube right on top of the head of the ultrasonic generator, as the ultrasonic strength decreases significantly away from the head of generator. Consult your user manual to identify the location of the generator head or do a test extraction to identify the position that produces the best RNA yield.*) Sonicate for 30 min at 22 °C (*Note: You may place the sonicator in a ventilation fume hood with its door closed to reduce the noise during operation*). After sonication, centrifuge at 12,000 xg for 5 min to pellet the fecal debris. Optional: Add 250 µl of isopropanol-diluted (1:1 dilution) AquaRemove to the crude lysate. Vortex and incubate at 22 °C for 5-10 min. Centrifuge again at 12,000 xg at 22 °C for 5 min to pellet the fecal debris.

2. Precipitate the DNA/RNA: Transfer 0.6 ml cleared lysate to a 1.5-ml tube and add 0.8 volume (0.48 ml) isopropanol. Vortex to mix. Centrifuge at 12,000 xg for 5 min to pellet the DNA/RNA. (Proteins remain in the isopropanol supernatant and can be recovered by precipitation in 4 vol of acetone or by dialysis). Decant to discard the supernatant. Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA/RNA pellet air-dry for 5-10 min.

3. Solubilize the DNA/RNA: Add 0.4 ml deionized water to the pellet, pipet and vortex to disperse the pellet. Incubate at 22 °C for 15 min and then centrifuge at 12,000 xg for 5-10 min to pellet any insoluble. Transfer the clear DNA/RNA solution to a new tube and store at -20 or -80 °C (*Note: Centrifuge the frozen DNA solution to remove any insoluble before its use in a PCR reaction*).
The expected RNA/DNA concentration is about 450 ng/ml. The total RNA/DNA yield from 0.5 ml of AquaStool preserved stool specimen (~50 mg of feces) is about 150-200 µg, of which about 1/2 – 1/3 is RNA.

5) PCR and RT-PCR Amplification

The most common application of fecal DNA and RNA is to determine if specific DNA or RNA sequences exist in the fecal specimen for the diagnosis of cancers, bacterial, viral, fugal, and parasitic infections; identification of transgenic animals; analysis of human and animal intestinal microbiome; and survey of wildlife animals. AquaStool-purified fecal DNA and RNA are suitable for various PCR and RT-PCR detections. The PCR and RT-PCR protocols provided here may be adjusted for your specific application.

1. DNase I digestion. AquaStool-extracted fecal RNA contains large amounts of sheared DNA. RT-PCR can be performed without removing the contaminating DNA, if appropriate primer pair is designed to avoid the amplification of genomic DNA sequence or produce different amplicon size. Otherwise, DNase I treatment is required prior to reverse transcription. To digest the DNA, 40 µl of the 400 µl of AquaStool-purified RNA is incubated with 0.5 ml of DNase I in its 1x buffer at 37 °C for 20-30 min. Following DNase digestion, the DNase may be removed with 4 µl of DNase Inactivation Reagent (Ambion # AM1906).

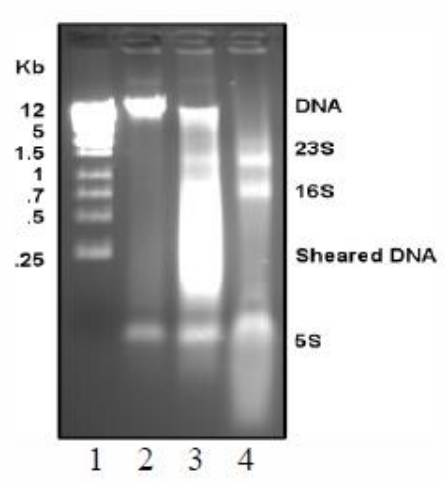

Fig. 1. AquaStool-purified fecal DNA and RNA. Aliquots (5 µl) of the extracted DNA and RNA were electrophoresized in a native 0.8% agarose gel.

1. DNA ladder.
2. DNA extracted by vortexing.
3. DNA/RNA extracted by sonication (The sheared DNA is ready for next generation sequencing. RNA contamination will not affect sequencing).
4. DNase I-treated RNA.

2. Reverse transcription. Anneal RT primer to its complimentary RNA by incubating 2 µl of 5 µM RT primer with 4 µl of DNase I-treated RNA and 12 µl of water at 80 °C for 4 min and then on ice. Following primer annealing, add 2 µl of 10x buffer, 0.2 µl of 25 mM dNTPs, and 0.5 µl of 100 U/µl MMLV Reverse Transcriptase to the mix and incubate at 42 °C for 60 min to synthesize the cDNA. Heat-inactivate the MMLV Reverse Transcriptase by incubating at 94 °C for 10 min.

3. PCR amplification. Assemble a 30 µl PCR reaction by mixing 3 µl of 10x PCR reaction buffer (with 2.5 mM $MgCl_2$), 0.3 µl of 25 mM dNTPs, 2 µl of 5 µM PCR primer pair, 25 µl of nuclease-free water, 0.3 µl of DNA polymerase, and 2.5 µl of the above RT reaction (*for RT-PCR*) or 0.5 µl of ~200 ng/µl AquaStool-purified DNA (*for PCR*). Run 30 cycles of PCR amplification.

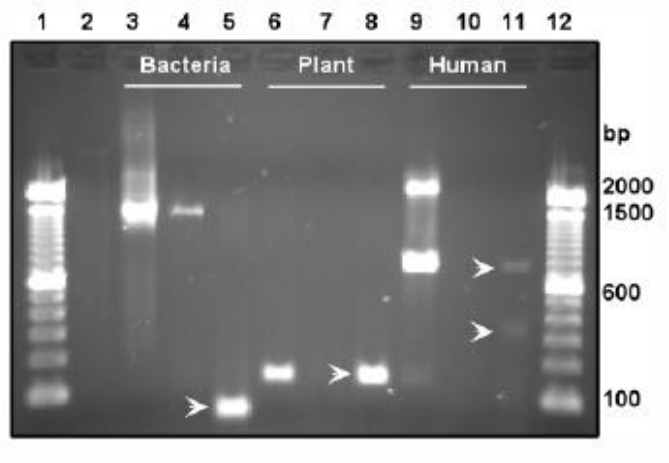

Fig. 2. PCR and RT-PCR amplification of fecal DNA and RNA.
Human fecal DNA and RNA were extracted with AquaStool. Bacterial, plant (food), and host DNA and RNA in the fecal specimen were analyzed by PCR and RT-PCR. Lane 1 and 12 are 100 bp DNA ladder. Lane 2 is no DNA/RNA negative control. Lane 3 (PCR), 4 (no RT control), and 5 (RT-PCR) were amplified with a bacterial primer pair. Lane 6 (PCR), 7 (no RT control), and 8 (RT-PCR) were amplified with a plant primer pair. Lane 9 (PCR), 10 (no RT control), and 11 (RT-PCR) were amplified with a human primer pair. Arrows point at RT-PCR products. The data indicates that it is possible to extract both fecal DNA and RNA with AquaStool for analyzing DNA and RNA biomarkers of host, food, and microbial origins.

C. Wood DNA Extraction

This protocol uses 500 µl of AquaStool and 125 µl of AquaRemove (#1208MT, not included, please order separately) to extract DNA from 50 mg of wood powder. DNA yield may vary (50 ng to 5 µg) by species, age, sampling location, sample storage conditions, etc. Fresh or frozen wood samples usually provide the best DNA yields.

1. Transfer ~50 mg of wood powder to a 1.6 or 2-ml microfuge tube. Add 50-100 mg of white sand (Sigma # 274739, white, 50+70 mesh) and 500 µl of AquaStool to the tube. Shake and vortex the inverted tube at top speed for 30-60 sec. Incubate at 22 °C for 30-60 min. Vortex again for 30-60 sec.

2. Secure the microfuge tube with a lid lock. Place the tube at the middle of a 5x16-well microfuge tube stand filled with cold tap water. Place the tube stand and sample in a microwave oven (900W and 2450MHz) and heat for 30 sec (pre-test a sample in your microwave oven to ensure the lid is not leaked after 30 sec of microwave heating).

3. Remove the tube stand from the microwave oven. Immediately vortex at top speed for 30-60 sec. (*Optional: Repeat Step 2 for another 30 sec of microwave heating may increase DNA yield*)

4. Centrifuge at 12,000 xg for 5 min to pellet the debris. Add 250 ml of isopropanoldiluted (1:1 dilution) AquaRemove. Vortex to mix well and centrifuge at 12,000 xg for 5 min.

5. Transfer as much lysate as possible (insert the pipette tip into the sand to withdraw the remaining lysate) to a new 1.6 ml microfuge tube. Centrifuge again to pellet any carried-over debris. Carefully transfer the clear lysate to a new tube. Add 0.8 vol of isopropanol to the clear lysate (e.g., add 400 µl isopropanol to 500 µl cleared lysate). Vortex to mix well and incubate at 22 °C for 5 min.

6. Centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then decant to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA/RNA pellet air-dry for 5-10 min. Add 50 µl of deionized water to the pellet, pipet and vortex to fully disperse the pellet.
Incubate at 65 °C for 15 min and then centrifuge at 12,000 xg for 5 min to pellet any insoluble. Transfer the clear DNA solution to a new tube and store at –20 °C (Centrifuge the frozen DNA solution to remove any insoluble before its use in a PCR reaction).

Frequently Asked Questions

Please read through these questions carefully. The answers provide additional helpful tips and useful information for the successful use of AquaStool.

1. How should I store the AquaStool kit?

AquaStool may be stored at 22 °C for 12 months. If AquaStool becomes precipitated when exposed to low temperature, you may incubate it at 37-50 °C for 15-20 min to redisolvethe reagent.

2. Why shouldn’t I use Bleach to disinfect AquaStool preserved fecal specimen?

AquaStool contains guanidine thiocyanate. It may react with Bleach (sodium hypochlorite) and release toxic gases.

3. How should I air-dry the mouse fecal samples?

Air-dried mouse fecal samples can be stored long term at room temperature for future genotype verification. To air-dry a mouse fecal pellet, incubate the fecal pellet in an opened microfuge tube on a dry heat bloc at 37 °C for 24 hours.

4. Why is my DNA/RNA solution showing a strong absorption below A260?

It is likely due to trace amount of guanidine salt contamination. If it interferes with your downstream applications, you may further purify the extracted DNA/RNA with a silica spin column (e.g., a plasmid miniprep column). Simply add an equal volume of 4 M GuHCl and 1M NaOAc (pH unadjusted, ~7.0) to your DNA/RNA solution (may contain the insoluble

pellet) and load it into the spin column, centrifuge to allow DNA/RNA binding to the silica membrane, wash the column with 0.6 ml 75% EtOH, and elute the DNA/RNA in 50 µl of deionized water or TE buffer.

5. My mouse transgene was not amplified well, how may I improve it?

You may try the following to improve mouse fecal DNA amplification:
(a) after freezing the fecal DNA solution at -20 °C, recentrifuge it to pellet and remove any insoluble, which may contain PCR inhibitors;
(b) reduce the amount of fecal DNA used per PCR reaction (i.e., try using 0.5, 0.25, 0.1 and 0.01 ml of extracted fecal DNA per PCR reaction);
(c) increase PCR cycles to 65;
(d) add 1 mM DTT and 0.1 mg/ml BSA to the PCR reaction;
(e) use a gel imager to visualize faint amplicon bands;
(f) use AquaRemove (#1208, order separately) with AquaStool to purify fecal DNA (see "Human fecal DNA/RNA extraction" for details); and
(g) further purify the fecal DNA with a silica spin column as outlined in #4 Question and Answer above.

Order Information, Shipping and Storage

Order#	Product	Quantity
7001MT	AquaStool™	1 ml
7030MT	AquaStool™	30 ml
shipped at RT ; store at RT		

Contact and Support

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