

AquaStool Instruction Manual

General Information

Description

Stool is an accessible and underutilized noninvasive source of biospecimen. Fecal DNA originated from the host, commensal bacteria, viruses, fungi, or parasites, and incompletely digested foods are molecular fingerprints of the host and its health. AquaStool™ is a multifunctional aqueous solution-based reagent for fecal DNA and RNA extraction and fecal PCR inhibitor removal. It may be used to extract fecal DNA for non-invasive genotyping of transgenic animals. Unlike tail/toe/ear snipping, fecal sampling is not limited by animal age, physiopathological condition, and sampling frequency. It is particularly useful for fecal DNA extraction to verify adult animals' genotype and transgene stability.

Specification

Product Name	AquaStool™ Kit
Product #	7001, 7030
Size	7001: 6 Extractions, 7030: 200 Extractions
Kit Contents	7001: 1 ml AquaStool Solution, User Manual 7030: 30 ml AquaStool Solution, User Manual
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at 4°C. Invert to mix well before dispensing.

Terms & Condition

Product Usage: For In Vitro Laboratory Research Use Only. NOT to be administered to humans or used for medical diagnosis.

Limited Product Warranty: We offer a LIMITED PRODUCT WARRANTY to our customers. This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by MultiTarget Pharmaceuticals. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

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

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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's fecal pellet. Invert or vortex the reagent bottle to mix the reagent well before dispensing.

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: Add 150 µl of AquaStool to each fecal pellet. Let the pellet soak in AquaStool solution for 15-30 min, use a pipette tip to break up the pellet if needed, and then vortex at top speed for 1-2 min to fully homogenize the fecal material. To improve bacterial DNA extraction, vortex the fecal sample in the presence of ~100 µg of white sand (Sigma # 274739, white, 50+70 mesh). Centrifuge at 14,000 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 (~100 µl) to a new 0.5-ml microfuge tube. Add 0.8 vol (~80 µl) of isopropanol and vortex for 1 min to mix the contents. Centrifuge at 14,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, then flip the tube to discard the ethanol solution. Be sure to rinse the lid of the tube as well, as it may catch some reagent. Repeat the 70% ethanol rinse 2 times. Place the tube upside down on a clean paper towel for 5-10 min to air-dry the DNA pellet. Add 100 µl of TE buffer or deionized water to the DNA pellet, pipette or vortex vigorously to suspend the DNA. Centrifuge at 14,000 xg for 10 min to pellet any insoluble material, which contains fecal PCR inhibitors, and then transfer the clear DNA/RNA solution to a new tube.

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

AquaGenomic Stool Protocol

AquaGenomic (#2030) is a nontoxic and non-corrosive reagent for DNA extraction. It may also be used to extract mouse fecal DNA and tail DNA for genotyping, with the benefit of using the lysate for PCR. This protocol uses 200 µl of AquaGenomic solution to extract DNA from a mouse fecal pellet.

1. Collect the fecal pellet

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.

2. Extract the DNA

Add 200 µl of AquaGenomic solution to each fecal pellet. Incubate at 85 °C for 20 min. (For tail DNA extraction, place the tissue into a microfuge tube preloaded with 100 µl of AquaGenomic solution containing 10 µg of Proteinase K. Incubate at 60 °C for 2 hrs and then at 95 °C for 10 minutes to inactivate the Proteinase K. Homogenize the tissue by vortexing or pipetting.)

3. Pellet the Debris

Centrifuge at 12,000 xg for 4 min to pellet the debris. Transfer the supernatant (~100 µl) to a new 0.5-ml microfuge tube. (*Note: To use the lysate for PCR, dilute an aliquot of the lysate with 9 vol of deionized water and use 0.5 µl of the diluted lysate in a 25-µl PCR reaction.*)

4. Pellet the DNA

Add 0.8 vol (~80 µl) of isopropanol and mix by vortexing for 30 sec. Centrifuge at 12,000 xg for 2 min to pellet the DNA. Flip the tube to discard the supernatant. Fill the microfuge tube with 70% ethanol by shooting the ethanol solution at the cap of the tube from a squirt bottle, and then flip to discard the ethanol. Repeat the 70% ethanol rinse 2 times. Air-dry the DNA pellet. Add 50 µl of TE buffer or deionized water, vortex vigorously to suspend the DNA. Centrifuge again for 2 min to pellet any insoluble and transfer the clear DNA solution to a new tube.

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 4 °C for 12 months. Invert to mix the reagent well before dispensing.

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 upon mixing if the AquaStool waste volume is sufficiently large.

3. Can I use AquaStool to extract DNA from other biospecimens?

Yes, for DNA extraction from cultured cells, simply add AquaStool solution to the cell pellet or the culture dish after removing the culture medium, and vortex to lyse the cells. For DNA extraction from animal tissues, such as tail snips, homogenize the tissue sample in AquaStool solution. After lysis and homogenization, follow the fecal DNA extraction protocol to recover the DNA from the cleared lysate.

4. How should I air-dry the fecal samples?

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

5. Do I need to ship mouse fecal samples in dry ice?

No, you can ship mouse fecal samples to other laboratories or genotyping facilities at ambient temperature, even in the summer, if they have been air-dried.

6. I had a very weak amplification, any tips?

You may try the following tips to improve fecal DNA amplification and detection. (a) You should re-centrifuge the DNA solution to pellet any insoluble material just before adding it to the PCR reaction, as some insoluble material may develop during storage. (b) You may use 45-65 PCR cycles for the amplification. (c) You may try adding 1 mM DTT to the PCR reaction, which helps re-activate the inactive polymerase. (d) You may try adding 0.1 mg/ml BSA to the PCR reaction, which may sequester residual PCR inhibitors. (e) Finally, a gel imager may be needed to detect the faint amplicon bands.