DATA SHEET

AquaStool Instruction Manual

General Information

Description

Stool is an accessible, abundant, under-utilized, and 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 health and disease conditions of the host. AquaStool is a multifunctional reagent. It extracts fecal DNA and removes PCR inhibitors that are abundant in fecal samples. The use of fecal DNA for genotyping transgenic animals not only improve the welfare of the animals but also allows the investigator to do genotyping without being limited by the animal's age and sampling frequency. AquaStool is especially useful for fecal DNA extraction from adult animals to verify their genotype and their transgene stability. AquaStool may also be used to extract DNA, RNA and proteins from other tissues, including tail snips. Please contact us for protocols, if you intend to use AquaStool to extract RNA and proteins.

Specification

Product Name	AquaStool™ Kit
Product #	7001, 7030
Size	7001: 6 Extractions, 7030: 200 Extractions
Kit Contents	7001: 1 ml AquaStool Solution, Instruction Manual 7030: 30 ml AquaStool Solution, Instruction Manual
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at 4°C. Vortex 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.

Patents, Trademarks & Copyrights

Mouse fecal DNA extraction and genotyping

AquaStool can 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. Furthermore, fecal pellets can be air-dried and conveniently stored at room temperature for later DNA extraction and genotype verification.

1. Fecal sample collection: Transfer a mouse to a clean cage (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.*). Put 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 on a 37 °C dry heat block with the tube opened for 24 hrs. The 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 or longer, 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.

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 60 sec 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!*). Amplify the transgene and a host gene as an internal control with 45-65 cycles of thermal cycles.

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. Vortex to mix the AquaStool suspension 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 volume is sufficiently large.

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

Yes, for extraction from cultured cells, simply add AquaStool solution to the cell pellet or the culture dish after removing the culture medium, and vortex the resulting cell lysate to extract DNA. For animal or plant tissues, homogenize the tissue in AquaStool solution to extract DNA.

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 another laboratory 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 a few things to improve fecal DNA amplification. First, you should centrifuge the DNA solution one more time to pellet the insoluble material just before taking it to a PCR reaction, as some insoluble material may develop during storage. Secondly, try using 45-65 PCR cycles for the amplification. Thirdly, try adding 1 mM DTT to the PCR reaction, which helps re-activate the inactive polymerase. Fourthly, try adding 0.1 mg/ml BSA to the PCR reaction, which may sequester residual PCR inhibitors. Finally, a gel imager may be needed to pick up faint amplicon bands that are difficult to see.