AquaGenomic Instruction Manual

General Information

Description

AquaGenomic™ is an aqueous reagent for DNA extraction. It may be used to extract DNA from all types of specimens, from bacteria to animal tissues. The extraction protocols are simple, fast, and scalable. AquaGenomic is nontoxic and its lysate may be used for PCR without further DNA purification. AquaGenomic is highly efficient in extracting DNA from dried specimen swabs. It enables the use of cotton swabs for specimen collection, transportation, and storage at room temperature, therefore, making dried specimen swabs ideal for low-complexity and low-cost biobanking, biosurveillance, and epidemiological research applications.

Specification

Product Name	AquaGenomic™ Kit
Product #	2001, 2030
Size	2001: For 10 minipreps from cultured cells 2030: For 300 mini, 30 midi, and 3 maxi preps from cultured cells
Kit Contents	2001: 1 ml AquaGenomic Solution, User Manual 2030: 30 ml AquaGenomic Solution, User Manual
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at RT (~22°C).

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.

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AquaGenomic Cell Protocol

This protocol can be used to prepare 5-10 µg of genomic DNA from 1-2 million cultured cells. For other sample sizes, use 100 µl of AquaGenomic Solution for each million nucleated cells.

1. Harvest the Cells

Pellet ~0.5-2 million cultured cells in a 1.5-ml microfuge tube by centrifugation at 12,000 xg for 60 sec. Aspirate or decant to discard the supernatant.

2. Extract the DNA

Add 100 µl of AquaGenomic to the cell pellet. Suspend and lyse the cells by vortex vigorously for 60 sec. (*Optional: Incubate at 75° C for 20 min for best DNA yield, DNase inactivation and RNA degradation.*)

3. Pellet the Debris

Centrifuge at 12,000 xg for 5 min to pellet the debris. (*Note: To use the lysate for PCR, dilute an aliquot of the lysate with 10-20 vol of water and then use 0.25 µl of the diluted lysate in a 25-µl PCR reaction.*)

4. Pellet the DNA

Transfer the clear lysate (~90 µl) to a new 0.5-ml microfuge tube. Add 0.8 vol (~72 µl) of 100% isopropanol and vortex for 60 sec to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, and then flip the tube to discard the ethanol solution. Repeat the 70% ethanol rinse once. 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 12,000 xg for 5 min to pellet any insoluble material, and transfer the clear DNA solution to a new tube.

AquaGenomic Tissue Protocol

This protocol may be used to extract DNA from tissues (including common research specimens, such as drosophila, mouse-tail snip, nematode, zebrafish, and plant tissues) by homogenization or by Proteinase K digestion in AquaGenomic solution. Approximately 10-20 µg of DNA can be extracted from 10 mg of animal tissue.

1. Harvest the Cells

Cut out a ~2 mm cube (~10 mg) of frozen or fresh tissue.

2. Extract the DNA

By homogenization: Homogenize the tissue in 200 µl of AquaGenomic. Move the pestle up and down slowly while vortexing at top speed to enhance homogenization. Alternatively, homogenize the tissue in 0.5-ml screw-capped tubes with a multichannel bead beater. After homogenization, add 1/10 volume (~10 µl) of isopropanol to the sample to reduce foaming (*do not add isopropanol prior to homogenization as it will neutralize AquaGenomic's cell lysis ability*), vortex and transfer the homogenate to a 1.5-ml microfuge tube. (*Optional: Incubate at 75° C for 20 min for best DNA yield, DNase inactivation and RNA degradation.*)

By Proteinase K digestion: Place the tissue (e.g., a mouse tail clip) in a microfuge tube preloaded with 200 µl of AquaGenomic containing 10 µg of Proteinase K. Incubate at 55 °C for >90 min to digest the tissue, and then at 95 °C for 10 min to inactivate the Proteinase K. The tissue is readily disintegrated by vortexing or pipetting.

3. Pellet the debris

Centrifuge at 12,000 xg for 5 min to pellet the debris. (*Note: To use the lysate for PCR, dilute an aliquot of the lysate with 10-20 vol of water and then use 0.25 µl of the diluted lysate in a 25-µl PCR reaction.*)

4. Pellet the DNA

Transfer the clear lysate (~180 µl) to a new 0.5-ml microfuge tube. Add 0.8 vol (~144 µl) of 100% isopropanol and vortex for 60 sec to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, and then flip the tube to discard the ethanol solution. Repeat the 70% ethanol rinse once. 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 12,000 xg for 5 min to pellet any insoluble material, and transfer the clear DNA solution to a new tube.

AquaGenomic Whole Blood Protocol

AquaGenomic may be used to extract total blood cellular and cell-free DNA from whole blood samples and its lysate may be used directly for PCR without further purification.

(a) To use lysate directly for PCR

1. Add 20 µl of AquaGenomic solution to each well in a 0.2-ml 96-well PCR plate.

2. Transfer 10 µl of well-mixed fresh or thawed whole blood to the AquaGenomic solution. Pipet up and down a few times to mix.

3. Incubate at 75 °C for 20 min in a PCR machine with heated lid. After the incubation, add 170 µl of water to the blood clot. Shake or pipet up and down a few times to mix.

4. To amplify the DNA, add 0.25 µl of the diluted lysate to 25 µl of PCR master mix and subject the reaction mix to 35-45 cycles of PCR amplification.

(b) To purify total blood DNA

1. Add 200 µl of AquaGenomic solution to a 0.5-ml tube.

2. Transfer 100 µl of well-mixed fresh or thawed whole blood to the AquaGenomic solution. Pipet up and down a few times to mix.

3. Incubate at 75 °C for 20 min. After the incubation, centrifuge the samples at 12,000 xg for 5 min. Transfer the clear lysate (~200 µl) to a new 0.5-ml microfuge tube.

4. Add 0.8 vol (~160 µl) of 100% isopropanol to the clear lysate and vortex for 60 sec to mix the contents. Centrifuge at 12,000 xg for 5-10 min at 22 °C to pellet the DNA. Decant to discard the supernatant. Carefully fill the tube with 70% ethanol from a squirt bottle, and then flip the tube to discard the ethanol solution. Repeat the ethanol rinse once.

5. Place the tube upside down on a clean paper towel for 5-10 min to air-dry the DNA pellet. Add 50 µl of TE buffer or deionized water to the DNA pellet, vortex vigorously to suspend the DNA.

AquaGenomic Saliva Protocol

Approximately 10-20 μg of genomic DNA can be obtained from 50 μl of saliva or a buccal swab or 200 μl of mouthwash, using one of the following methods.

1. Harvest the Cells

(a) Saliva. Swirl and rub your tongue against the inside of your cheek and gum for ~5-10 times. Carefully spit the saliva into a clean weight boat or a 15-ml conical tube.
(b) Swab. Use a swab to rub the inside of your cheek and gum for ~5-10 times and let it soak up the saliva. Air-dry the swab in its pouch.
(c) Mouthwash. Swirl and rub your tongue against the inside of your cheek and gum for ~5-10 times. Rinse the mouth with 10-20 ml of Scope mouthwash and spit it into a 50-ml conical tube.

2. Extract the DNA

(a) Saliva. Transfer 50 μl of saliva to a microfuge tube preloaded with 100 μl of AquaGenomic solution and incubate at 75 °C for 20 min.
(b) Swab. Cut off the tip of the swab into a 1.5-ml microfuge tube. Add 300 μl of AquaGenomic solution and incubate at 75 °C for 20 min. Use a 1-ml pipet tip to smash the swab 10 times to the bottom of the tube to squeeze out of the liquid.
(c) Mouthwash. Centrifuge 200 μl of mouthwash at 10,000 xg for 5 min to pellet the buccal cells and discard the supernatant. Add 200 μl of AquaGenomic solution. Vortex to mix well.

3. Pellet the Debris

Centrifuge at 12,000 xg for 5 min to pellet the debris. (*Note: To use the lysate for PCR, dilute an aliquot of the lysate with 10-20 vol of water and then use 0.25 μl of the diluted lysate in a 25-μl PCR reaction.*)

4. Pellet the DNA

Transfer the clear lysate (~100 μl) to a new 0.5-ml microfuge tube. Add 0.8 vol (~80 μl) of 100% isopropanol and vortex to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, and then flip the tube to discard the ethanol solution. Repeat the 70% ethanol rinse once. 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.

AquaGenomic Microbe Protocol

This protocol can be used to prepare 10-20 µg of DNA from 1 ml overnight microbial culture. For other preparation scales, use 100 µl of AquaGenomic Solution for each milliliter of overnight culture.

1. Harvest the Cells

Centrifuge 1 ml overnight bacterial culture at 12,000xg for 60 sec to pellet the cells. Aspirate or decant to discard the supernatant.

2. Extract the DNA

For Gram-negative bacteria: Add 100 µl of AquaGenomic Solution to the cell pellet. Suspend the cells by vortexing vigorously for 30 sec. (*Optional: Incubate at 75° C for 20 min for best DNA yield, DNase inactivation and RNA degradation*.). Alternatively, homogenize the samples in AquaGenomic in 0.5-ml screw capped tubes with a multichannel bead beater.

For Gram-positive bacteria or yeast: Treat the bacterial or yeast cells with lysozyme or lyticase (not supplied) according the enzyme manufactures' instruction. Add ~50 µg of 0.5-1 mm glass beads and 100 µl of AquaGenomic Solution containing 100 µg/ml Proteinase K to the sample. Incubate at 55° C for 60 min and then at 95 °C for 10 min to inactivate the Proteinase K.

3. Pellet the Debris

Centrifuge at 12,000 xg for 5 min to pellet the debris. (*Note: To use the lysate for PCR, dilute an aliquot of the lysate with 10-20 vol of water and then use 0.25 µl of the diluted lysate in a 25-µl PCR reaction.*)

4. Pellet the DNA

Transfer the clear lysate (~90 µl) to a new 0.5-ml microfuge tube. Add 0.8 vol (~72 µl) of 100% isopropanol and vortex for 60 sec to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, and then flip the tube to discard the ethanol solution. Repeat the 70% ethanol rinse once. 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 12,000 xg for 5 min to pellet any insoluble material, and transfer the clear DNA solution to a new tube.

AquaGenomic Stool and Soil Protocol

This protocol uses 200 µl of AquaGenomic Solution to prepare 5-10 µg of DNA from 15 mg of feces. AquaPrecipi solution (Item # 3015, not included) is generally required to purify fecal and soil DNA and remove PCR inhibitors. However, for mouse fecal DNA extraction and genotyping, AquaPrecipi purification may not be necessary.

1. Harvest the Cells

Weigh out 15 mg of wet feces (~10 mg of dry fecal pellet or a mouse fecal pellet) or 30 mg of soil in a 1.5-ml microfuge tube.

2. Extract the DNA

Add 200 µl of AquaGenomic solution to the sample. For dry fecal sample, let it soak in AquaGenomic solution until it is rehydrated. Homogenize the sample with a microfuge pestle or vortex vigorously for 1-2 min. Alternatively, homogenize the samples in AquaGenomic in 0.5-ml screw capped tubes with a multichannel bead beater. Incubate the sample at 75 °C for 20 min. If mitochondrial DNA extraction is desired, add Proteinase K to AquaGenomic to 100 µg/ml. Incubate at 55 °C for 60 min to digest the mitochondria and then at 95 °C for 15 min to inactivate the Proteinase K.

3. Pellet the Debris

Vortex vigorously for 60 sec and centrifuge at 12,000 xg for 5 min to pellet the debris. Transfer the clear lysate (~100 µl) to a new 0.5-ml microfuge tube (*Note: The lysates of most fecal and soil samples cannot be used directly in PCR reactions as they contain large amounts of PCR inhibitors. AquaPrecipi is required for the removal of these fecal and soil PCR inhibitors in the next step.).*

4. Pellet the DNA

Add 0.5 vol (~50 µl) of AquaPrecipi (#3015, order separately) and 0.5 vol (~50 µl) of 95-100% of ethanol. Vortex for 60 sec and centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, and then flip the tube to discard the ethanol solution. Repeat the 70% ethanol rinse once. 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 12,000 xg for 10 min to pellet any insoluble material, which contains residual PCR inhibitors, and transfer the clear DNA solution to a new tube.

AquaGenomic Swab Protocol

Cotton swabs are commonly used in forensic evidence collection. However, existing DNA extraction methods can recover only ~100-500 ng of DNA from a dried specimen swab as the majority of DNA remains entrapped within the cotton matrix. With AquaGenomic, ~5-8 μg of DNA can be extracted from a dried specimen swab. It is possible to use cotton swabs for specimen collection, transportation, storage, and DNA extraction for low-complexity and low-cost biobanking, biosurveillance, and epidemiological research applications.

1. Collect the specimen. Use a cotton swab to soak up the specimen (~100-200 μl), such as blood, saliva, mucus, semen, feces, cultured mammalian or bacteria cells, homogenized animal or plant tissues, or any other potential sources of biospecimens. Air-dry the specimen swab at ambient temperature (20-50 °C) for >24 hours. The dried specimen swabs can be shipped at ambient temperature and then stored at room temperature in sealed paper envelopes or plastic bags with desiccant for many years.

2. Extract the proteins and small molecules (dried blood swabs). Cut off the specimen swab tip into a 1.5-ml microfuge tube. Add 400 μl deionized water or buffer and soak the swab for >30 min. Use a 1-ml pipet tip to smash the swab ~10 times and press it to the bottom of the tube to squeeze out the solution. Transfer as much liquid as possible (~300 μl) to a new 1.5-ml microfuge tube for analysis of plasma proteins or small molecules.

3. Extract the DNA. Add 300 μl AquaGenomic to the 1.5-ml tube containing the wet swab from Step 2 or a new dried specimen swab. Incubate at 22 °C for 1 hr and then at 75-85 °C for 20 min. Use a 1-ml blue pipet tip to smash the swab ~10 times and press it to the bottom of the tube to squeeze out the solution. Alternatively, homogenize the samples in AquaGenomic in 0.5-ml screw capped tubes with a multichannel bead beater. Transfer as much liquid as possible (~200 μl) to a new 0.5-ml microfuge tube or use a microfuge spin bucket to recover all the lysate from the swab to maximize the DNA yield (*Optional: To use the crude lysate for PCR, dilute an aliquot of the crude lysate with 10-20 vol of deionized water and use 0.25 μl of the diluted lysate in a 25-μl PCR reaction*).

4. Purify the DNA. Centrifuge at 10,000 xg for 5 min to pellet any debris in the crude lysate. Transfer the clear lysate (~200 μl) to a 0.5-ml tube and mix with 1 vol of isopropanol (~200 μl). Centrifuge at 10,000 xg for 5 min to pellet the DNA. Flip the tube to discard the isopropanol supernatant as completely as possible. Gently shoot 50% isopropanol from a squirt bottle to fill up the tube. Flip the tube to discard the isopropanol rinse as completely as possible. Tap and place the tube upside down on a clean paper towel to remove residual solution. Air-dry the DNA pellet for 5-10 min. Add 100 μl of deionized water to the DNA pellet and let it rehydrate for >15 min. Vortex or pipet up and down to solubilize the DNA.

AquaGenomic Avian Blood Protocol

This protocol may be used to prepare about 50-100 µg of genomic DNA from 10 µl of avian blood sample stored in 100 µl of Queen's lysis buffer (10 mM Tris, 10 mM NaCl, 10 mM EDTA, 1% n-lauroylsarcosine, pH 7.5), using 200 µl of AquaGenomic solution and 100 µl of AquaRemove solution (order separately, #1208).

1. Collect the blood sample

Avian blood samples are routinely collected in Queen's lysis buffer at a ratio of 1 volume to 9-10 volumes, and stored at room temperature until DNA extraction.

2. Extract the DNA

Add 200 µl of AquaGenomic to 100 µl of lysed avian blood sample in Queen's lysis buffer. Vortex vigorously for 60 sec. (*Optional: Incubate at 75° C for 20 min for best DNA yield, DNase inactivation and RNA degradation*.)

3. Pellet the Debris

Add 100 µl of isopropanol diluted AquaRemove (order separately, #1208. *Note: Dilute the AquaRemove solution with an equal volume of isopropanol before use*.) and vortex vigorously for 60 sec to mix well. Centrifuge at 12,000 xg for 5 min to pellet the debris.

4. Pellet the DNA

Transfer the clear lysate (~400 µl) to a new 1.5-ml microfuge tube. Add 0.8 vol (~320 µl) of 100% isopropanol and vortex for 60 sec to mix well. Centrifuge at 12,000 xg for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, and then flip the tube to discard the ethanol solution. Repeat the 70% ethanol rinse once. 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.

Frequently Asked Questions

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

1. Do I need to keep AquaGenomic in the freezer?

No, AquaGenomic Solution is stable at room temperature (~22 °C) for >1 year.

2. Does AquaGenomic Solution contain Proteinase K?

No. AquaGenomic can be used to extract DNA from most cells and tissues without needing protease digestion. However, adding Proteinase K (50 µg/ml) to AquaGenomic solution can increase DNA yield and is required for mitochondrial DNA extraction. You may homogenize the sample in AquaGenomic containing Proteinase K, incubate it at 55 °C for 1-2 hrs and then at 95 °C for 10-15 min to inactivate the Proteinase K.

3. AquaGenomic sounds like a "green" product, any particular precaution?

AquaGenomic is nontoxic and non-corrosive. It contains no phenol, chloroform, guanidine HCl, or other harmful chemicals commonly used for DNA extraction. There is no particular precaution while using AquaGenomic; you just need to follow standard good laboratory practice in handling laboratory chemicals.

4. I am worried about cross-contamination using homogenizers, any tips?

Between uses, you may wash the homogenizer with soap and running water, soak it in 10% bleach for ~5 min, and then rinse it with running deionized water. If you still feel uneasy, you may use Proteinase K digestion without using a homogenizer, or use a multichannel bead beater for homogenization in screw-capped tubes.

5. Do I have to use the lysate immediately for PCR?

No, you may store the lysate at 4 °C until analysis. If the lysate has been incubated at 85 °C for 20 min, it may even be left at room temperature until analysis.

6. I got a weak PCR amplification using the lysate directly, how may I improve it?

You may try a few things to optimize the amplification: a) try use different amount of lysate for the PCR, form 0.25 µl undiluted lysate to 20x diluted lysate, b) add 0.1 mg/ml BSA to the PCR reaction, c) add 1 mg/ml DTT to the PCR reaction, and d) increase the PCR cycle number to 45 cycles.