AquaGenomic Solution

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Contents

General Information

Specification

AquaGenomic™ is a multifunctional aqueous solution for the isolation and purification of genomic DNA. In one step, the single solution functions to lyse cells, extract the DNA, and precipitate cell debris. It can be used to isolate genomic DNA from various sources, e.g. from cultured cells, microbials, animal or plant tissues. The protocols are simple, fast and scalable. Approximately 5 - 10 µg DNA can be isolated from 1 - 2 million cultured cells, 1 ml of bacterial cultures, 5 - 10 µg of animal tissues, or 10 - 20 mg of plant tissues.

Frequently Asked Questions

Please read this section carefully before using the AquaGenomic™ Solution. The answers to the following questions provide additional tips for the successful use of AquaGenomic™ (page 16).

Terms & Conditions

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

Limited Product Warranty:
Product warranty is limited to our liability to replacement of this product. All other warranties, expressed or implied, including but not limited to any implied warranties of merchantability or fitness for a particular purpose, are excluded and do not apply. 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.

Protocols

Equipment and Reagents by the User

All chemicals should be handeled using Good Laboratory Practice (GLP). Always wear rubber gloves and laboratory coat when handling AquaGenomic™ Solution.

Equipment and Reagents for all AquaGenomic™ protocols:

- Standard equipment for handling samples
- Centrifuge capable of generation appropriate g-force
- Appropriate centrifugation tubes (e.g. 1.5 ml microcentrifuge tubes)
- Appropriate rack for holding the tubes
- 70% ethanol
- 100% isopropanol
- Resuspension fluid (e.g. Aqua dest., TE buffer)
- Recommended: Vortexer

Additionally, for the 96-WELL PLATE Protocol:

- 96-deep-well plate
- 96-well plate centrifuge
- Recommended: Plate shaker

Equipment and Reagents for standard agarose gel electrophoresis:

- Horizontal gel electrophoresis system
- Agarose
- Markers

We recommend to use MoBiTec's high-quality special agaroses & DNA markers and its Dark Reader™ transilluminator.

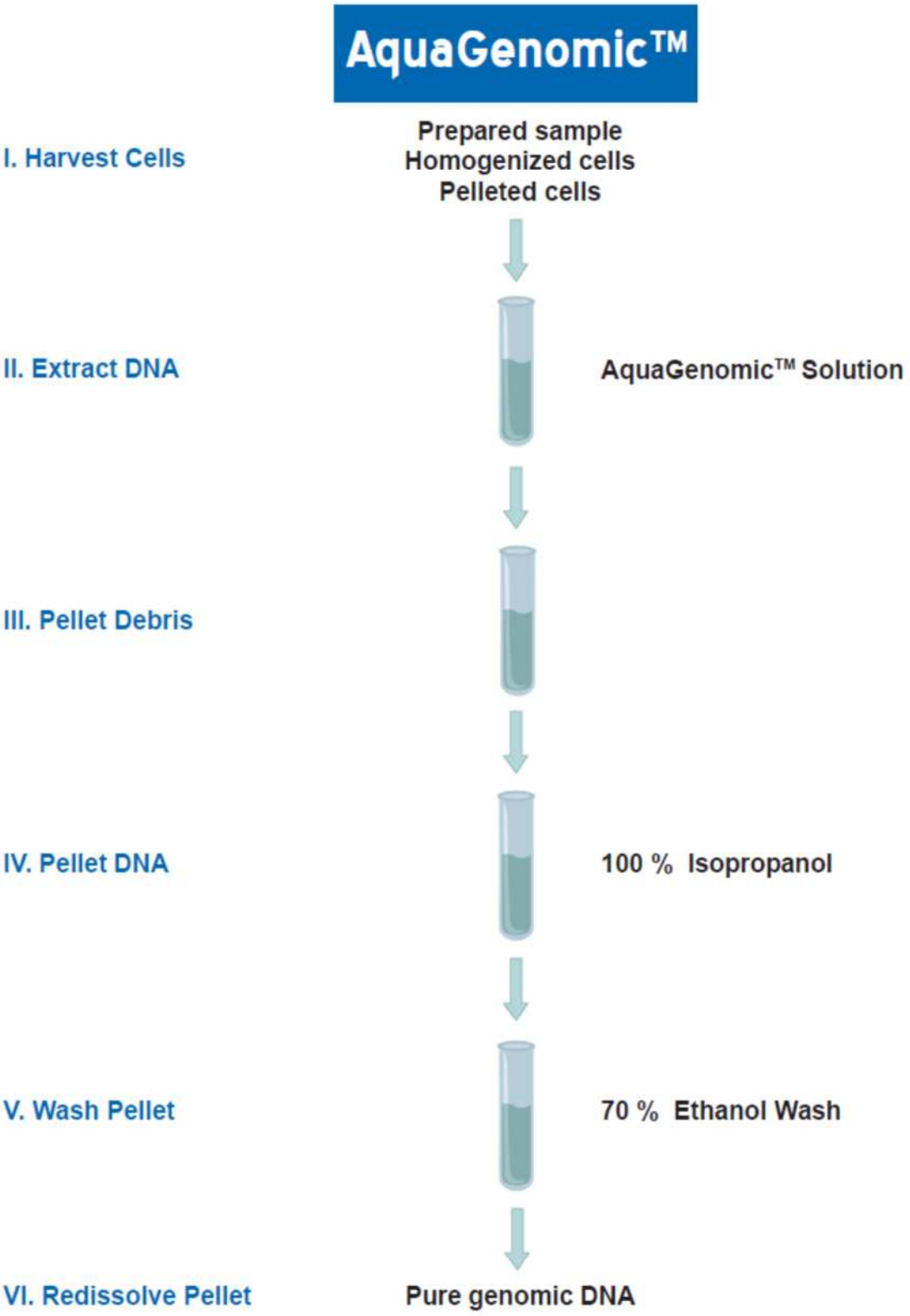
Order#	Product	Quantity
04004G	MoBiTec Agarose LE	500 g
04043G	MoBiTec Ladder 1 kb extended	250 µg
AZS7567	SYBR® Green I nucleic acid gel stain *10,000X concentrate in DMSO*	1 ml
AZS11494	SYBR® Gold nucleic acid gel stain *10,000X concentrate in DMSO*	500 µl
DR-195M	30 x 46 cm DR Transilluminator	
DR-88X	22 x 25 cm DR transilluminator	
DR-46B	15 x 19 cm DR Transilluminator	

Equipment and Reagents for UV/VIS spectrometry:

- UV spectrophotometer & accessories

We recommend to use the Qubit™ fluorometer as a complete, integrated solution to all your lab quantitation tasks. By pairing the power and simplicity of the Qubit™ Fluorometer with the unmatched performance of the Quant-iT™ Assay Kits, a major source of uncertainty from your workflow has been eliminated.

Order#	Product	Quantity
AZQ32857	Qubit® fluorometer	Each
AZQ32851	Quant-iT™ dsDNA HS Assay Kit, 100 assays *0.2-100 ng* *for use with the Qubit® fluorometer*	Kit
AZQ32854	Quant-iT™ dsDNA HS Assay Kit, 500 assays *0.2-100 ng* *for use with the Qubit® fluorometer*	Kit
AZQ32856	Qubit™ assay tubes *set of 500*	Set


AquaGenomic™
Prepared sample
Homogenized cells
Pelleted cells
I. Harvest Cells
II. Extract DNA
AquaGenomic™ Solution
III. Pellet Debris
IV. Pellet DNA
100 % Isopropanol
V. Wash Pellet
70 % Ethanol Wash
VI. Redissolve Pellet
Pure genomic DNA

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 x g for 60 sec. Aspirate or decant to discard the supernatant.

2. Extract the DNA

Add 100 µl of AquaGenomic™ Solution to the cell pellet. Suspend and lyse the cells by vortex vigorously for 60 sec. Incubate at 75 °C for 15 - 30 min.

Note: *The lysate may be used directly in PCR reaction at a final concentration of 1%, i.e., use 0.5 µl of lysate in a 50 µl PCR reaction.*

3. Pellet the Debris

Vortex vigorously for 60 sec and centrifuge at 12,000 x g for 5 min to pellet the debris.

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 x g 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. 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 x g 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, and zebrafish, etc.) 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 100 µl of AquaGenomic solution. Move the pestle up and down slowly while vortexing at maximal speed to enhance homogenization. After homogenization, add 1/10 volume (~10 µl) of isopropanol to the sample to reduce foaming, vortex, and transfer the homogenate to a 1.5 ml microfuge tube. Incubate at 75 °C for 15 - 30 min.

By Proteinase K digestion: Place the tissue in a microfuge tube preloaded with 100 µl of AquaGenomic™ containing 10 µg of Proteinase K. Incubate at 65 °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.

Note: *The lysate may be used directly in PCR reaction at a final concentration of 1%, i.e., use 0.5 µl of lysate in a 50 µl PCR reaction.)*

3. Pellet the debris

Vortex vigorously for 60 sec and centrifuge at 12,000 x g for 5 min to pellet the debris.

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 x g 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. 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 x g for 5 min to pellet any insoluble material, and transfer the clear DNA solution to a new tube.

AquaGenomic™ Tail Protocol

AquaGenomic™ is a nontoxic and non-corrosive aqueous solution based reagent for DNA extraction. The lysate may be used directly for PCR, making AquaGenomic the simplest method for tail DNA extraction and genotyping. This protocol uses 50 µl of AquaGenomic™ Solution to extract DNA from a 2 mm tail snip.

1. Harvest the Tissue

Clip off ~2 mm long tail from mouse < 21 days old. Place the tissue into a microfuge tube preloaded with 50 µl of AquaGenomic™ Solution containing 10 µg of Proteinase K (e.g., add 2 µl of 5 mg/ml Proteinase K stock solution to 50 µl of AquaGenomic solution just before the extraction).

2. Extract the DNA

Incubate at 65 °C for 2 hrs (or overnight) and then at 95 °C for 10 min to inactivate the Proteinase K. The tissue is readily disintegrated by vortexing or pipetting vigorously.

Note: *The lysate may be used directly in PCR reaction at a final concentration of 1%, i.e., use 0.5 µl of lysate in a 50 µl PCR reaction. Or you may dilute the lysate with 1 vol of deionized water and then use 0.5 µl of the diluted lysate in a 25 µl PCR reaction. The lysate may be used immediately or stored at -20 °C until PCR analysis. A 40 to 45-cycle of PCR reaction generally produces a strong amplification signal.*

3. Pellet the Debris

Centrifuge at 12,000 x g for 4 min to pellet the debris. Transfer the supernatant (~40 µl) to a new 0.5 ml microfuge tube.

4. Pellet the DNA

Add 1 vol (~40 µl) of isopropanol and mix by vortexing for 30 sec. Centrifuge at 12,000 x g 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, pipette up-and-down and 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.

AquaGenomic™ Dry Blood Spot Protocol

This protocol may be used to extract DNA from dry blood spot (DBS) on blood card punches with diameters of approximately 3 - 6 mm (1/8 – 1/4 inch). It may also be used to extract DNA from dry blood stains on cloths, papers, swabs, and other fibrous backings. The DNA yield is approximately 500 - 1,000 ng per 6 mm punch, a critical factor for increasing the detection sensitivity.

1. Place a 6 mm DBS punch (or two 3 mm punches) in a 1.5 ml microfuge tube. Add 200 µl of AquaGenomic™ Solution to the tube.
2. Centrifuge at 12,000 x g for 1 min to sink the disc into the AquaGenomic™ Solution.
3. Incubate at 75 °C for 30 min. After the 30 min incubation, use a 1 ml pipette tip on a pipette to strike the disc against the bottom of the tube 10 - 20 times to smash it and squeeze the solution out of the matrix (or if available, use a motorized microtube pestle or a multitube bead beater for processing large number of samples).
4. Centrifuge at 12,000 x g for 5 min. Transfer the clear lysate (~120 µl) to a new 0.5 ml microfuge tube.

Note: *The lysate may be used directly in PCR reaction at a final concentration of 1%, i.e., use 0.5 µl of lysate in a 50 µl PCR reaction.*

5. Add 1 vol (~120 µl) of 100% isopropanol to the clear lysate and vortex for 60 sec to mix the contents. Centrifuge at 12,000 x g 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, then flip the tube to discard the ethanol solution. Repeat the 70% ethanol rinse once.
6. 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™ Whole Blood Protocol

This protocol may be used for high throughput blood DNA extraction and genotyping in 96-well plate format. You may use 25 µl of AquaGenomic™ to extract DNA from 25 µl of whole blood. Additionally, the AquaGenomic™ crude lysate can be used directly for PCR, which makes the analysis much simpler and faster. Furthermore, AquaGenomic™ is nontoxic while other genomic DNA extraction kits use the toxic and hazardous guanidine hydrochloride. The benefit of using AquaGenomic™ to the health of lab personnel and the environment is obvious. Not to mention that in contrast to other blood DNA extraction methods, AquaGenomic™ recovers total blood DNA, including cell-free circulating or viral DNA, and provides the opportunity to use the same blood samples for the detection of both cellular and plasma-born targets.

1. Add 25 µl of AquaGenomic™ Solution to each well in a 0.2 ml 96-well PCR plate.
2. Transfer 25 µl of well-mixed fresh or thawed whole blood to the AquaGenomic™ Solution in each well. Pipet up and down a few times to mix.
3. Incubate at 75 °C for 20 min in a PCR machine with heated lid (to extract viral, bacterial, fungal, and mitochondrial DNA in the blood, you may need to add Proteinase K to the AquaGenomic™ Solution, incubate the lysate at 65 °C for 1 - 2 hrs, and inactivate the Proteinase K at 95 °C for 15 min). After the incubation, the crude lysate may be used immediately for PCR or stored at -20 °C until analysis.
4. To amplify the DNA, mix 0.5 µl of the crude lysate with 50 µl of PCR master mix and subject the reaction mix to 40 - 45 cycles of PCR amplification.

Important: *The final concentration of the lysate in the PCR reaction should be no more than 1%. For pipetting accuracy, you may dilute the lysate with water before transferring it to the PCR mix, e.g., dilute the lysate with 1 vol of water and use 0.5 µl of the diluted lysate in a 25 µl PCR reaction.*

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™. Vortex to mix well and incubate at 75 °C for 15 - 30 min.

(b) Swab. Cut off the tip of the swab into a 1.5 ml microfuge tube containing 200 µl of AquaGenomic™. Vortex and incubate at 75 °C for 15 - 30 min. After the incubation, use a 1 ml pipette tip to strike the swab against the bottom of the tube 10 - 20 times to squeeze the solution out of the swab (or if available, use a motorized microtube pestle or a multitube bead beater for processing large number of samples).

(c) Mouthwash. Centrifuge 200 µl of mouthwash at 10,000 x g for 5 min to pellet the buccal cells and discard the supernatant. Add 200 µl of AquaGenomic™. Vortex to mix well and incubate at 75 °C for 15 - 30 min.

Note: The lysate may be used directly in PCR reaction at a final concentration of 1%, i.e., use 0.5 µl of lysate in a 50 µl PCR reaction.

3. Pellet the Debris

Vortex and centrifuge at 12,000 x g for 5 min to pellet the debris.

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 to mix well. Centrifuge at 12,000 x g for 5 min to pellet the DNA. Decant to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle, 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 x g for 5 min to pellet any insoluble material, and transfer the clear DNA solution to a new tube.

AquaGenomic™ Plant Protocol

This protocol may be used to isolate ~10 - 20 µg of genomic DNA from 20 mg of plant tissues, using 200 µl AquaGenomic™ Solution.

1. Harvest the Cells

Weigh out ~20 mg of fresh or frozen plant tissue. Cut the tissue into small pieces. Place them in 200 µl of AquaGenomic™ Solution in a pestle-and-tube homogenizer.

2. Extract the DNA

Slowly move the pestle up and down while vortexing at maximal speed to enhance homogenization. After homogenization, add 1/10 volume (~10 µl) of isopropanol to the sample to reduce foaming, vortex, and transfer the homogenate to a 1.5 ml microfuge tube. Incubate at 75 °C for 15 - 30 min.

Note: *The lysate may be used directly in PCR reaction at a final concentration of 1%, i.e., use 0.5 µl of lysate in a 50 µl PCR reaction.*

4. Pellet the debris

Vortex vigorously for 60 sec and centrifuge at 12,000 x g for 5 min to pellet the debris.

5. 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 x g 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. 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 x g for 5 min to pellet any insoluble material, and transfer the clear DNA solution to a new tube.

AquaGenomic™ Microbe Protocol

This protocol can be used to prepare ~10 - 20 µg of genomic 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 12,000 x g 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. Incubate the sample at 75 °C for 15 - 30 min to lyse the cells (For some strains, cell lysis and DNA yield may be enhanced by incubating at 90 °C for 30 min).
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 65 °C for 60 min and then at 95 °C for 10 min to inactivate the Proteinase K.

Note: *The lysate may be used directly in PCR reaction at a final concentration of 1%, i.e., use 0.5 µl of lysate in a 50 µl PCR reaction.*

3. Pellet the Debris

Vortex vigorously for 60 sec and centrifuge at 12,000 x g for 5 min to pellet the debris.

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 x g 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. 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 x g 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 150 µl of AquaGenomic™ Solution to prepare 5 - 10 µg of DNA from 15 mg of feces. AquaPrecipi Solution (Item # 3015MT, not included) is required to purify fecal DNA and remove PCR inhibitors.

1. Harvest the Cells

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

2. Extract the DNA

Add 150 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. Incubate the sample at 75 °C for 15 - 30 min. If mitochondrial DNA extraction is desired, add Proteinase K to AquaGenomic™ to 100 µg/ml. Incubate at 60 °C for 60 min to digest the mitochondria and then at 95 °C for 10 min to inactivate the Proteinase K.

3. Pellet the Debris

Vortex vigorously for 60 sec and centrifuge at 12,000 x g for 5 min to pellet the debris. Transfer the clear lysate (~100 µl) to a new 0.5 ml microfuge tube.

Note: *Unlike the lysate from other specimen sources, the lysate of fecal and soil samples cannot be used directly in PCR reactions as they contain large amounts of fecal and soil 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 and 0.5 vol (~50 µl) of 95 - 100% of ethanol. Vortex for 60 sec and centrifuge at 12,000 x g 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. 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 x g for 10 min to pellet any insoluble material, which contains residual PCR inhibitors, and transfer the clear DNA solution to a new tube.

Frequently Asked Questions

Please read this section carefully before using the AquaGenomic™ Solution. The answers to the following questions provide additional tips for the successful use of AquaGenomic™.

1. **Do I need to store the AquaGenomic™ kit in the refrigerator or freezer?**
   No, AquaGenomic™ Solution is stable at room temperature (~22° C) for 12 months. Invert the bottle a few times to mix the solution well before use.

2. **Should I keep the solutions and samples on ice while carrying out the isolation?**
   No. Most of the isolation steps should be performed at room temperature (~22 °C). For DNA isolation from animal cells, cell lysis at 60 °C may increase the yield by ~25%. For DNA isolation from cells with a cell wall, such as bacterial and plant cells, the cell lysis should be carried out at 60 - 90 °C for 15 - 30 min.

3. **Does AquaGenomic™ Solution contain Proteinase K?**
   No. AquaGenomic™ Solution to extract genomic DNA from cells and tissues can be used without proteinase K digestion. This reduces the time required for genomic DNA isolation from solid tissues from a full day to only ~20 minutes. However, if mitochondrial DNA recovery is desired, you will need to add Proteinase K to AquaGenomic™ Solution to 100 µg/ml and incubate the sample at 65 °C for 1 - 2 hrs during cell lysis and then at 95 for 10 min to inactivate the Proteinase K.

4. **What type of homogenizer do you recommend for using with AquaGenomic™?**
   Pestle-and-tube homogenizers produce more homogeneous samples. However, if you do not have a homogenizer, you can chop the tissue with a pair of tweezers, and then vortex it in AquaGenomic™ Solution to extract the DNA. Another option is to use the 60 °C extraction method, which does not require a homogenizer but a few hours of 60 °C incubation. At the end of incubation, you may use glass or ceramic beads to disrupt the tissue. If beads are not available, a microfuge tube pestle or a pipette tip can be used to chop the tissue.

5. **Does AquaGenomic™ Solution contain RNase A?**
   No. AquaGenomic™ Solution can remove most RNA contaminants. Trace amounts of RNA would not interfere with most genomic DNA applications. If complete RNA removal is desired, RNase A (not included; Order No. RIBA25) can be added to AquaGenomic™ Solution at 25 mg/ml and incubated the lysate at 37 - 65 °C for 5 min to degrade the RNA.

6. **Why do I need to purchase a separate reagent for DNA isolation from stool or soil?**
   Due to the presence of large amount of enzyme inhibitors in feces and soil, DNA isolation from these samples are particularly challenging. Precipitation of the DNA with isopropanol cannot remove some of the inhibitors and a specially formulated AquaPrecipi Solution (Order No. 3015MT) is required for selective precipitation of the DNA to remove these enzyme inhibitors.

7. **Can I use AquaGenomic™ to isolate DNA from frozen blood?**
   Yes. The AquaGenomic™ Blood Protocol uses low concentration of AquaGenomic™ Solution to lyse RBC (1 volume of AquaGenomic™ Solution to 20 volumes of whole blood). This RBC lysis method does not depend on a functional RBC membrane and, therefore, can be used for lysis of RBC from either fresh or frozen blood.

8. **I am concerned about cross-contamination using homogenizers, any tip?**
   Yes. Between uses, you should thoroughly wash the homogenizer with soap and running water, soak it in 10% bleach for ~5 minutes, and then rinse it with deionized water. This will prevent cross-contamination of the genomic DNA. If you still feel uneasy, you can use the 60 °C extraction and the bead milling method to disrupt the tissues.

9. **What genomic DNA yield can I expect using AquaGenomic™?**
   Approximately 10 µg DNA can be obtained from 1 - 2 million cultured cells, 300 - 400 µl of whole blood, 1 ml of overnight microbial culture, 5 - 10 mg of animal tissues, or 10 - 20 mg of plant tissues. DNA yield is dependent on the number of nucleated cells in the sample and may vary at different cell cycles and for different cell types.

10. **How do I adjust the amount of AquaGenomic™ Solution based on the amount of starting material?**
    Using too much cells/tissues and too little AquaGenomic™ Solution could result in less clean DNA preparation. As a general guideline, you should use 100 µl of AquaGenomic™ Solution for 1 - 2 million cultured cells, 500 µl of whole mammalian blood, 1 ml of overnight microbial cultures, half a buccal swab, or 5 mg of animal or plant tissues.

11. **How pure is the genomic DNA isolated by AquaGenomic™?**
    Typical A_{260}/A_{280} of AquaGenomic™ purified DNA is 1.6 - 1.8. The isolated genomic DNA is free from most cellular contaminants and enzyme inhibitors. However, there may be some RNA contamination, if RNase A treatment is not included.

12. **What QC tests do you use to certify your products?**
    Each lot is tested to ensure its performance and reliability in isolating genomic DNA. The isolated DNA (a) should have an $A_{260}/A_{280} > 1.6$, and (b) should be readily digested with restriction enzymes.

13. **AquaGenomic™ sounds like a "green" product, any particular cautions?**
    Yes, AquaGenomic™ is nontoxic and non-corrosive. It contains no phenol, chloroform, guanidine hydrochloride, and 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.

14. **Do you have more tips for using the lysate directly in PCR?**
    Make sure that the final concentration of the lysate in the PCR reaction must be no more than 1%. If your amplification fails, the first thing to look at is pipetting accuracy. For accurate pipetting, you may dilute the lysate with water and then use the diluted lysate in the PCR, e.g., dilute the lysate with 1 vol of water and use 0.5 µl of the diluted lysate in a 25 µl (or 30 µl, to compensate possible pipetting error) PCR reaction. If the tissue debris interferes accurate pipetting, you may centrifuge the crude lysate to pellet the debris. Finally, you may need to use 45 - 55 PCR cycles to obtain a strong amplification signal.

Order Information, Shipping and Storage

Order#	Product	Quantity
2001MT	AquaGenomic™ Solution Trial Kit	Kit
2010MT	AquaGenomic™ Solution 30 preps	Kit
2030MT	AquaGenomic™ Solution 300 preps	Kit
3015MT	AquaPrecipi Solution	15 ml
shipped at RT; store at RT		

Contact and Support

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MoBiTec in your area: Find your local distributor at www.mobitec.com