

AquaPlasmid Instruction Manual

General Information

Description

AquaPlasmid™ is a novel aqueous solution-based reagent for isolation and purification of plasmid DNA. It yields ~5-10 µg of plasmid DNA from a milliliter of overnight bacterial culture. The isolated DNA has an A260/A280 ratio of 1.8-2.0 and A260/A230 ratio of 2.0-2.2. The extraction protocol is as simple as alkaline lysis and it can be carried out using an inexpensive personal picofuge. AquaPlasmid reagent does not contain guanidine hydrochloride, a not readily biodegradable chemical found in most plasmid DNA purification kits.

Specification

Product Name	AquaPlasmid™ Kit
Product #	1001, 1015
Size	1001: 0.5 ml for 10 minipreps 1015: 15 ml for 300 minipreps, or 30 midipreps, or 3 maxipreps
Kit Contents	1001: 0.5 ml AquaPlasmid solution, 1 ml Aqualysis solution, User Manual 1015: 15 ml AquaPlasmid solution, 30 ml AquaLysis solution, User Manual
MSDS	Available at www.aquaplasmid.com
Storage	Store tightly capped at 22°C.

Terms & Conditions

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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AquaPlasmid Miniprep Protocol

This miniprep protocol yields ~10-20 µg of plasmid DNA from 2 ml overnight bacterial cultures, using 100 µl of AquaLysis solution and 50 µl of AquaPlasmid solution. For other culture volumes, scale up or down using 50 µl of AquaLysis solution and 25 µl of AquaPlasmid solution for each milliliter of culture volume.

1. Harvest the Cells

Transfer 1.9 ml of overnight bacterial culture to a 2-ml microfuge tube. Centrifuge at 3,500-14,000 xg for 1 min at room temperature (~22° C) to pellet the bacteria. Aspirate to remove the culture medium as completely as possible.

2. Lyse the Cells

Add 80 µl of deionized water to the cell pellet. Vortex vigorously to fully resuspend the cells (~100 µl). Add 100 µl of AquaLysis solution to the cell suspension. Vortex to mix the contents well. Incubate at room temperature for 1-5 min.

3. Remove the Debris

Add 50 µl of AquaPlasmid solution to the crude lysate. Touch-vortex (a few seconds on and then a few seconds off) at top speed to mix the contents well. Incubate at room temperature for 1-5 min. Centrifuge at 3,500-14,000 xg for 5 min to pellet the debris.

4. Pellet the Plasmid DNA

Transfer 210 µl of the clear lysate to a 0.5-ml microfuge tube. Add 1/3 vol (70 µl) of isopropanol to the lysate, i.e., the final isopropanol concentration is ~25%. Vortex to mix well. Centrifuge at 3,500-14,000 xg at room temperature for 5 min to pellet the plasmid DNA. Flip the tube to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle (*be sure to rinse the inside of the lid as well*), and then flip the tube to discard the ethanol solution. Repeat the ethanol rinse once. Flip the tube forcefully a few times and blot it on a paper towel to remove residual ethanol. Place the tube upside down on the paper towel to air-dry the DNA pellet (near invisible) for 5-10 min. Add 50 µl of deionized water to the DNA pellet, vortex to solubilize the DNA.

AquaPlasmid Midiprep Protocol

This midiprep protocol yields ~100-200 µg of plasmid DNA from 20 ml overnight bacterial cultures, using 1 ml of AquaLysis solution and 0.5 ml of AquaPlasmid solution. For other culture volumes, scale up or down using 50 µl of AquaLysis solution and 25 µl of AquaPlasmid solution for each milliliter of culture volume.

1. Harvest the Cells

Centrifuge 20 ml of overnight bacterial culture at 3,500-14,000 xg for 5 min at room temperature (~22° C) to pellet the bacteria. Aspirate to remove the culture medium as completely as possible.

2. Lyse the Cells

Add 0.8 ml of deionized water to the cell pellet. Vortex vigorously to fully resuspend the cells (~1 ml). Add 1 ml of AquaLysis solution to each tube. Vortex to mix the contents well. Incubate at room temperature for 1-5 min.

3. Remove the Debris

Add 0.5 ml of AquaPlasmid solution to the crude lysate. Touch-vortex (a few seconds on and then a few seconds off) at top speed to mix the contents well. Incubate at room temperature for 1-5 min. Centrifuge at 3,500-14,000 xg for 5 min to pellet the debris.

4. Pellet the Plasmid DNA

Transfer the clear lysate (2x 1.2 ml) into two 1.5-ml microfuge tubes. Add 1/3 vol (0.4 ml) of isopropanol to each tube, i.e., the final isopropanol concentration is ~25%. Vortex to mix well. Centrifuge at 3,500-14,000 xg at room temperature for 5 min to pellet the plasmid DNA. Flip the tube to discard the supernatant. Fill the tube with 70% ethanol from a squirt bottle (*be sure to rinse the inside of the lid as well*), and then flip the tube to discard the ethanol solution. Repeat the ethanol rinse once. Flip the tube forcefully a few times and blot it on a paper towel to remove residual ethanol. Place the tube upside down on the paper towel to air-dry the DNA pellet (near invisible) for 5-10 min. Add 125 µl of deionized water to each DNA pellet, vortex and/or pipette to solubilize the DNA. Centrifuge at 3,500-14,000 xg for 2 min to pellet any insoluble and transfer and combine the DNA solution to a new microfuge tube.

AquaPlasmid Maxiprep Protocol

This maxiprep protocol yields ~1-2 mg of plasmid DNA from 200 ml overnight bacterial cultures (*For highest plasmid DNA yield, use Terrific Broth or other rich media.*), using 10 ml of AquaLysis solution and 5 ml of AquaPlasmid solution. For other culture volumes, scale up or down using 50 µl of AquaLysis solution and 25 µl of AquaPlasmid solution for each milliliter of culture volume.

1. Harvest the Cells

Centrifuge 200 ml of overnight bacterial culture at 3,500-14,000 xg for 5 min at room temperature (~22° C) to pellet the bacteria. Aspirate to remove the culture medium as completely as possible.

2. Lyse the Cells

Add 8 ml of deionized water to the cell pellet. Vortex vigorously to fully resuspend the cells (~10 ml). Add 10 ml of AquaLysis solution to each tube. Rotate and flick tube or vortex to mix the contents well. Incubate at room temperature for 5 min.

3. Remove the Debris

Add 5 ml of AquaPlasmid solution to the crude lysate. Shake the sample vigorously to break up the aggregates into fine pieces and vortex at top speed to mix the contents well. Incubate at room temperature for 5 min. Centrifuge at 3,500-14,000 xg for 10 min to pellet the debris.

4. Pellet the Plasmid DNA

Transfer the clear lysate (24 ml) into a 50-ml centrifuge tube. Add 1/3 vol (8 ml) of isopropanol to lysate, i.e., the final isopropanol concentration is ~25%. Vortex to mix well. Centrifuge at 3,500-14,000 xg at room temperature for 10 min to pellet the plasmid DNA. Decant the tube to discard the supernatant. Add ~25 ml of 70% ethanol (*Do not disturb the DNA pellet*), rotate the ethanol solution to rinse the entire interior of the tube, and then decant the tube to discard the ethanol solution. Repeat the ethanol rinse once. Place the tube upside down on a clean sheet of paper towel to air-dry the DNA pellet (near invisible) for 5-10 min. Add 1 ml of deionized water to the DNA pellet, vortex and/or pipette to solubilize the DNA.

AquaPlasmid 96-Well Plate Protocol

This protocol may be used to prepare plasmid minipreps in 96-well plates with bucket rotors in a plate centrifuge or even a speed-vac. It may be adapted to liquid handling robots for high-throughput minipreps. The plasmid DNA yield is ~5-10 µg from 1 ml overnight bacterial cultures.

1. Harvest the Cells

Grow the bacteria in 1 ml culture in 96 deep-well (1-2 ml) plate(s) in a 37° C shaker overnight. The plate should be sealed with a microporus tape. Centrifuge the overnight bacterial culture in bucket rotors at allowed maximal speed for 15 min at room temperature (~22° C) to pellet the bacteria. Aspirate to remove the culture medium as completely as possible.

2. Lyse the Cells

Add 40 µl of deionized water to the cell pellet. Vortex or pipet up and down to resuspend the cells. Add 50 µl of AquaLysis solution to the cell suspension. Vortex or pipette vigorously to mix the contents well. Incubate at room temperature for 5 min.

3. Remove the Debris

Add 25 µl of AquaPlasmid Solution to each well. Vortex or pipette up and down 10-15 times to mix the contents. Incubate at room temperature for 1-5 min. Centrifuge at allowed maximal speed for 15 min at room temperature to pellet the debris.

4. Pellet the Plasmid DNA

Carefully transfer 120 µl of clear lysate from each well to corresponding well in a standard 96-well (200 µl) plate. Add 40 µl of isopropanol to each well. Shake the plate on a plate shaker for 2-3 min to mix the contents. Centrifuge the plate at allowed maximal speed for 15 min at room temperature to pellet the plasmid DNA. Flip the plate to discard the supernatant. Carefully fill the wells with 70% ethanol from a squirt bottle, and then flip the plate to discard the ethanol solution. Repeat the ethanol rinse once. Flip the plate forcefully a few times and blot it on a paper towel to remove residual ethanol. Place the plate upside down on the paper towel to air-dry the DNA pellet for 5-10 min. Add 50 µl deionized water to each well, vortex or pipette up and down to solubilize the DNA. Seal and store the DNA solution at 4° C or at -20° C for long-term storage.

Frequently Asked Questions

Please read through these questions before using AquaPlasmid kit. The answers provide additional tips and information for the successful use of AquaPlasmid.

1. Do I need to store the AquaPlasmid kit at 4 or –20 °C?

No, AquaPlasmid solution is stable at room temperature (~22 °C) for >1 year. However, if the room temperature is below 18 °C, some precipitation may occur in the AquaLysis solution, which can be re-solubilized by incubating at 37 °C for a few minutes.

2. How should I scale up and down the reagents for other starting culture volumes?

The recommended scaling base is “1 ml culture – 40 µl water – 50 µl AquaLysis – 25 µl AquaPlasmid.” For example, you may pellet the bacteria in 4 ml overnight culture, use 160 µl of water to suspend the bacterial pellet, add 200 µl of AquaLysis to lyse the cells, and add 100 µl of AquaPlasmid to neutralize the crude lysate.

3. Does AquaPlasmid contain RNase A?

No, AquaPlasmid solution does not contain RNase A. You may use AquaPlasmid purified plasmid DNA directly for in vitro transcription.

4. Can I use a low-speed centrifuge to do the minipreps?

Yes, you don't need a high-speed centrifuge to use the AquaPlasmid methods. You may even use a personal picofuge (1-6 minipreps) or a standard speed-vac (96-well plate HTP minipreps) for all the centrifugation steps.

5. Should I use centrifugation to rinse the DNA pellet?

No, it is not necessary. Gently shoot 70% ethanol solution into the tube along the sidewall away from the DNA pellet to fill up the tube, and then flip the tube to discard the ethanol solution. Repeat the ethanol rinse one time. For midi or maxiprep, make sure that the DNA pellet remains attached at the bottom of the tube before decanting to discard the ethanol solution.

6. How pure is the AquaPlasmid isolated plasmid DNA?

The plasmid DNA is essentially free of cellular impurities and other enzyme inhibitors. Plasmid DNA isolated by AquaPlasmid usually has an A260/A280 ratio of 1.8-2.0 and A260/A230 ratio of 2.0-2.2.