AquaPlasmid Instruction Manual

General Information

Description

AquaPlasmid™ is a multifunctional aqueous reagent for plasmid DNA purification without using columns. This single solution completes cell suspension, cell lysis, cell debris removal and plasmid DNA extraction in a single tube. AquaPlasmid is nontoxic. The multifunctionality makes AquaPlasmid the most economic ($0.66/miniprep) and environment-friendly (nontoxic) plasmid purification product on the market. The isolated plasmid DNA is suitable for all downstream applications, including automated DNA sequencing.

Specification

Product Name	AquaPlasmid™ Solution
Product #	1001, 1015, 1030, 1060
Size	1001: 1 ml AquaPlasmid for 5 minipreps 1015: 15 ml AquaPlasmid for 75 minipreps 1030: 30 ml AquaPlasmid for 150 minipreps 1060: 60 ml AquaPlasmid for 300 minipreps
MSDS	Available at www. multitargetpharm.com
Storage	Store tightly capped at 22°C. Incubate at 37°C to solubilize precipitates developed during transit/storage at low temperatures.

Terms & Conditions

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

Warranties and Liabilities: MultiTarget Pharmaceuticals accepts no responsibility and shall not be held liable for any loss, damage, expense, consequential, or accidental damage, including damage to property, person, or premises arising out of the use, the results of use, or the inability to use these products. MultiTarget Pharmaceuticals MAKES NO WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE.

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AquaPlasmid Miniprep Protocol

(1.5 ml LB culture yields 10-20 µg of plasmid.)

1. Harvest the Cells

Transfer 1.5 ml of overnight bacterial culture to a 1.7-ml microfuge tube. Centrifuge at 14,000 xg for 1 min at 22 °C to pellet the bacteria. Aspirate or flip the tube forcefully a few times to remove the culture medium as completely as possible.

2. Lyse the Cells

Add 200 µl of AquaPlasmid solution to the cell pellet. Immediately vortex for 10-20 sec to fully suspend the cells. Incubate at 22 °C for 5 min to lyse the cells. After the incubation, touch-vortex (1-2 sec on and 1-2 sec off) at top speed twice (*IMPORTANT: Do not over-vortex or it could cause genomic DNA contamination*).

3. Remove the Debris

Incubate the crude lysate at -20 °C for 5 min or on ice for 10 min to induce precipitation. Centrifuge at 14,000 xg for 5 min at 22 °C to pellet the cell debris (*centrifuge for 10 min at 4 °C may produce tighter debris pellet*).

4. Pellet the Plasmid DNA

Transfer the clear lysate (~200 µl) to a clean 0.5-ml microfuge tube. Add 0.5 vol (~100 µl) of isopropanol to the lysate (*do not use more than 0.7 vol of isopropanol or it may increase small RNA contamination*). Touch-vortex at top-speed 5-10 times to mix well. Centrifuge at 14,000 xg for 5 min at 22 °C to pellet the plasmid DNA. Flip the tube (*you may hold 6-10 tubes between your thumb and index finger at the same time*) forcefully a few times to discard the supernatant.

5. Rinse the Plasmid DNA

Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then flip the tube forcefully to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA pellet air-dry for 5-10 min. Add 50 µl of deionized water (or TE buffer) to the DNA pellet. Vortex to fully suspend the DNA pellet and incubate at 22 °C for 5 min. Centrifuge at 14,000 xg for 2 min to pellet any insoluble. Optional: Transfer the DNA solution to a new tube.

AquaPlasmid Midiprep Protocol

(15 ml LB culture yields 100-200 µg of plasmid DNA.)

1. Harvest the Cells

Transfer 2x 1.9 ml overnight bacterial culture to two 2-ml microfuge tubes and centrifuge at 14,000 xg for 1 min at 22 °C. Aspirate or flip the tube forcefully a few times to remove the culture medium as completely as possible. Repeat pelleting the bacteria from 1.9 ml culture in the same tube for 4 times for a total 7.5 ml culture per tube.

2. Lyse the Cells

Add 1 ml of AquaPlasmid solution to each tube. Immediately vortex at top speed for 20-30 sec to fully suspend the cells. Incubate at 22 °C for 10 min to lyse the cells. After the incubation, touch-vortex (1-2 sec on and 1-2 sec off) at top speed twice (*IMPORTANT: Do not over-vortex or it could cause genomic DNA contamination*).

3. Remove the Debris

Incubate the crude lysate at -20 °C for 5 min or on ice for 10 min to induce precipitation. Centrifuge at 14,000 xg for 5 min at 22 °C to pellet the cellular debris (*centrifuge for 10 min at 4℃ may produce tighter debris pellet*).

4. Pellet the Plasmid DNA

Transfer the clear lysate to two 1.7-ml microfuge tubes *(~0.8 ml each; be careful not to transfer any cellular debris; if necessary centrifuge the clear lysate again to remove any carried-over debris*). Add 0.5 vol (~0. 4 ml) of isopropanol to each tube (*do not use more than 0.7 vol of isopropanol or it may increase small RNA contamination*). Touch-vortex at top-speed 5-10 times to mix well. Centrifuge at 14,000 xg at 22 °C for 5 min to pellet the plasmid DNA. Flip the tube forcefully a few times to discard the supernatant.

5. Rinse the Plasmid DNA

Gently fill up the tube and its lid with 70% ethanol from a squirt bottle and then flip the tube forcefully to discard the ethanol solution. Repeat the ethanol rinse once. Tap the tube on a paper towel to remove residual ethanol and let the DNA pellet air-dry for 5-10 min. Add 200 µl of deionized water (or TE buffer) to each DNA pellet. Vortex to fully suspend the DNA pellet and incubate at 22 °C for 5 min. Centrifuge at 14,000 xg for 2 min to pellet any insoluble. Transfer the DNA solution to a new tube.

AquaPlasmid Maxiprep Protocol

(150 ml LB yields 1-2 mg of plasmid DNA.)

1. Harvest the Cells

Centrifuge 150 ml of overnight bacterial culture at 14,000 xg for 2 min at 22 °C to pellet the bacteria. Aspirate or decant to remove the culture medium partially and leave behind ~20 ml of medium. Vortex to resuspend the cells and transfer the cell suspension to a 50-ml high-speed centrifuge tube. Centrifuge again to pellet the cells. Aspirate or decant to remove the medium as completely as possible.

2. Lyse the Cells

Add 20 ml of AquaPlasmid solution to the cell pellet. Immediately vortex to fully suspend the cells. Incubate at room temperature for 15 min to lyse the cells. After the incubation, touch-vortex (1-2 sec on and 1-2 sec off) at top speed twice (*IMPORTANT: Do not over-vortex or it could cause genomic DNA contamination*).

3. Remove the Debris

Incubate the crude lysate at -20 °C for 5-10 min or on ice for 15 min to induce precipitation. Centrifuge at 14,000 xg for 15 min at 22 °C to pellet the cellular debris (*centrifuge for 15 min at 4 °C may produce tighter debris pellet*).

4. Pellet the Plasmid DNA

Transfer the clear lysate (~18 ml) to a new 50-ml centrifuge tube. Centrifuge the clear lysate for 5 min to remove any carried-over debris. Pour the clear lysate to a new 50-ml centrifuge tube. Add 0.5 vol (~9 ml) of isopropanol to the clear lysate (*do not use more than 0.7 vol of isopropanol or it may increase small RNA contamination*). Touch-vortex at top-speed 5-10 times to mix well. Centrifuge at 14,000 xg at 22 °C for 10 min to pellet the plasmid DNA. Flip the tube forcefully a few times to discard the supernatant.

5. Rinse the Plasmid DNA

Gently shoot 20-25 ml of 70% ethanol into the tube from a squirt bottle. Roll the tube to rinse the entire interior of the tube and then decant the tube to discard the ethanol solution. Repeat the ethanol rinse once. Flip the tube forcefully a few times and tap it on a paper towel to remove residual ethanol. Let the DNA pellet air-dry for 5-10 min. Add 1 ml of deionized water (or TE buffer) to the DNA pellet. Pipet and vortex to suspend the DNA pellet. Incubate at 22 °C for 10 min to solubilize the DNA. Centrifuge at 14,000 xg for 5 min to pellet any insoluble and transfer the clear DNA solution to a new microfuge tube.

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 °C?

No, AquaPlasmid solution should be stored at room temperature (~22 °C). If the temperature is below 18 °C, precipitates may develop. If so, incubate the solution at 37-55 °C for a few minutes and vortex to re-solubilize it before use.

2. How does AquaPlasmid work?

AquaPlasmid combines the functions of traditional P1 buffer (cell suspension), P2 buffer (cell lysis), N3 buffer (debris removal), and silica column (plasmid DNA purification) in a single solution. It lyses the cells without denaturing the DNA, extracts the plasmid DNA while keeping other cellular contaminants in the cell debris. The plasmid DNA is subsequently precipitated from the clear lysate with isopropanol.

3. Does AquaPlasmid contain guanidine salts?

No, AquaPlasmid does not contain guanidine salts that are required for column-based purification. Guanidine salts are not biodegradable. When mixed with Bleach, they can release toxic and mutagenic fumes into the environment, which may be harmful to lab workers and aquatic lives.

4. How should I scale up and down the reagent for other culture volumes?

We recommend using 200 µl of AquaPlasmid for each 1.5 ml of overnight bacterial culture. However, if the cell density is too high, it may result in poor cell lysis, incomplete debris removal and decreased DNA yield. An indication of cell density exceeding the capacity of AquaPlasmid is a large DNA pellet following isopropanol precipitation. In that case, you should use 300 µl of AquaPlasmid for each 1.5 ml of bacterial culture or use 200 µl of AquaPlasmid for each ml of culture.

5. Can I use AquaPlasmid to extract plasmid DNA from *endA1* strains?

We recommend AquaPlasmid only for plasmid extraction from *E. coli* strains carrying the *endA1* mutation, such as TOP10, DH5a, XL-1 Blue, JM109, and SURE, etc. If the genotype is unknown, you should incubate an aliquot of the purified DNA in a restriction enzyme buffer at 37 °C for 12 hours to confirm there is no DNA degradation. If the DNA is degraded, you may try incubating the purified DNA at 75-85 °C for 30 min to inactivate the contaminating nucleases.

MATERIAL SAFETY DATA SHEET (MSDS)

TRADE NAME: AquaPlasmid™ Solution
DATE OF ISSUE: March 1, 2015

SECTION I: PRODUCT AND MANUFACTURER INFORMATION

TRADE NAME: AquaPlasmid™ Solution

MultiTarget Pharmaceuticals, LLC
5050 Edison Ave Ste 214, Colorado Springs, CO 80915, USA
Telephone: 1-801-769-6586
DATE PREPARED: 03-01-2015

SECTION II: COMPOSITION / INFORMATION ON INGREDIENTS

SYNONYMS: AquaPlasmid™, plasmid DNA isolation solution
CHEMICAL CHARACTERIZATION: Proprietary aqueous solution of non-hazardous chemicals.
HAZARDOUS COMPONENT: None.

SECTION III: HAZARD IDENTIFICATION

May be harmful if swallowed. May cause irritation. Avoid breathing vapors, or dusts. Avoid contact with eyes, skin, and clothes. Wash thoroughly after handling. Keep container closed.

SECTION IV: FIRST AID MEASURES

IF EYE CONTACT: Immediately flush eyes with copious amounts of water for at least 15 minutes. Assure adequate flushing of eyes by separating the eyelids with fingers.
IF SKIN CONTACT: Immediately wash skin with soap and copious amounts of water.
IF SWALLOWED: Wash out mouth with water provided person is conscious. Call a physician.

SECTION V: FIREFIGHTING MEASURES

EXTINGUISHING MEDIA: Use extinguishing media appropriate to surrounding fire conditions.
SPECIAL FIREFIGHTING PROCEDURES: Wear self-contained breathing apparatus and protective clothing to prevent contact with eyes and skin.

SECTION VI: ACCIDENTAL RELEASE MEASURES

PRECAUTIONARY MEASURES: Wear self-contained breathing apparatus, chemical safety goggles, rubber boots, and heavy rubber gloves.
CLEAN-UP PROCEDURES: Absorb on sand or vermiculite and place in closed container for disposal. Ventilate area and wash spill site after material pick-up is complete.

SECTION VII: HANDLING AND STORAGE

STORAGE: Store tightly closed at 20-25 °C.

SECTION VIII: EXPOSURE CONTROLS AND PERSONAL PROTECTION

Chemical safety goggles. Rubber gloves. Safety shower and eye bath. Wash thoroughly after handling. Do not get in eyes, on skin, or on clothing.

SECTION IX: PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL STATE / FORM: Liquid
COLOR: Colorless
ODOR: None
pH: (20 °C) 8-9
VISCOSITY: (20 °C) N/A
MELTING POINT: N/A

BOILING POINT: N/A
IGNITION TEMPERATURE: N/A
FLASHPOINT: N/A
EXPLOSION LEVEL: N/A
VAPOR PRESSURE: (20 °C) N/A
SPECIFIC GRAVITY: (20 °C) N/A
SOLUBILITY IN WATER: (20 °C) Soluble

SECTION X: STABILITY AND REACTIVITY
SUBSTANCES TO BE AVOIDED: N/A
HAZARDOUS, COMBUSTION, OR DECOMPOSITION PRODUCTS: N/A

SECTION XI: TOXICOLOGICAL INFORMATION
TOXICITY DATA: N/A
INHALATION: May be harmful by inhalation.
EYE CONTACT: May cause eye irritation.
SKIN CONTACT: May cause skin irritation, may be harmful by skin absorption.
INGESTION: May be harmful if swallowed.
PROLONGED EXPOSURE: N/A
CHRONIC EFFECTS: N/A
RTECS NUMBER: N/A
ADDITIONAL INFORMATION: THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED. Additional harmful properties cannot be ruled out. The product should be handled with the normal caution accorded chemicals.

SECTION XII: ECOLOGICAL INFORMATION
N/A

SECTION XIII: DISPOSAL CONSIDERATIONS
There are no uniform regulations for the disposal of chemicals or residues. Dispose of container and unused contents in accordance with federal, state and local requirements.

SECTION XIV: TRANSPORT INFORMATION
DOT: None of the components are regulated.

SECTION XV: REGULATORY INFORMATION
SARA: None of the components are regulated.

SECTION XVI: OTHER INFORMATION
DATE OF PREPARATION: March 1, 2015.
DISCLAIMER: For research use only. The above information is believe to be correct but does not purport to be all inclusive and should be used only as a guide. MultiTarget Pharmaceuticals shall not be held liable for any damages or other consequences resulting from handling or from contact with the above product.