Quantum Prep®
Plasmid Maxiprep Kit

Catalog #
732-6130

BIO-RAD

732-6370	**AquaPure RNA Isolation Kit,** for cultured cells, animal and plant tissues, gram-negative bacteria; processes up to 100 (0.5–10 mg) animal or plant tissue preps, 100 cultured cell preps (1–2 x 10^6 cells/prep), or 100 x 0.5 ml bacterial cell cultures per kit
732-6371	**AquaPure RNA Blood Kit,** for human and mammalian whole blood and bone marrow; processes up to 100 x 0.3 ml whole blood samples per kit
732-6372	**RBC Lysis Buffer,** 100 ml
732-6348	**Proteinase K (20 mg/ml),** 175 µl
732-6349	**RNase A Solution,** 250 µl
732-6390	**Master Blaster™ RNA Extraction Reagent,** 100 ml

DH5α and DH10B are trademarks of Invitrogen. XL1-Blue is a trademark of Stratagene.

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Section 1. Introduction

1.1 Overview

The Quantum Prep plasmid maxiprep kit utilizes the patented DNA binding matrix found in the Quantum Prep plasmid mini and midiprep kits to yield milligram quantities of ultrapure plasmid DNA. This maxiprep protocol has been optimized for the isolation of high copy number plasmids grown in 250 ml of rich media such as Terrific Broth or 2x YT to a density of up to approximately 8 OD/ml. Luria broth (LB) cultures can be up to 500 ml with a density of 4 OD/ml. Larger cultures may require a proportional increase in solution volumes for efficient lysis. Yields of 1 to 3 mg of plasmid DNA can be obtained with high copy number plasmids. This DNA can be used for all types of applications, including automated fluorescent sequencing and transfection.

1.2 Contents

The Quantum Prep plasmid maxiprep kit contains reagents sufficient for 10 plasmid maxipreps.

165 ml	Maxiprep resuspension solution
250 ml	Cell lysis solution
165 ml	Neutralization solution
110 ml	Quantum Prep matrix
270 ml	Maxiprep wash buffer
10	Maxiprep spin baskets
5 ml	5 M NaCl

Materials required but not supplied: ethanol and 50 ml centrifuge tubes. Additional maxiprep wash buffer will be required if following recommendations for EndA$^+$ strains (see Section 2.1).

1.3 Storage and Stability

All components are guaranteed for 12 months from the date of purchase when stored at room temperature and used as described in this manual.

Section 2. Protocol

Various factors can influence the yield of plasmid DNA. These include vector copy number, insert DNA, host strain, growth conditions, and media. As mentioned above, this maxiprep protocol was designed to give the maximal yield of plasmid DNA for medium size culture volumes. This procedure was optimized using cultures grown to densities of 7–8 OD/ml in 250 ml of Terrific Broth. Luria Broth cultures can be grown to a density of 4 OD/ml in volumes up to 500 ml. Larger cultures will require a proportional increase in solution volume for efficient lysis.

The use of a host strain which contains a mutation in the endonuclease I gene (*endA*), such as JM109, DH5α, DH10B, or XL 1-Blue, is highly recommended. Isolation of DNA from strains containing active endonuclease I gene product (such as HB101 and MC1061) may result in samples which contain trace amounts of nuclease. These

strains should be avoided if the goal is to obtain DNA of the highest quality. Note the recommendations below for using this procedure with EndA$^+$ strains.

2.1 Recommendations for Best Results

A precipitate may form in the lysis or neutralization solutions when colder ambient temperatures are encountered in winter shipping conditions, cool laboratory temperatures, or factory storage conditions.

This precipitation will not affect performance of the product. If a precipitate is observed, warm the bottles to 37°C prior to use to redissolve the precipitate, then use at room temperature.

- Add 95–100% ethanol to the maxiprep wash buffer before first use.
- Eluting with water or TE heated to 70°C may provide an improved yield.
- DNA may be used directly after the elution step without ethanol precipitation. If additional purity or concentration is desired, the sample may be precipitated as described.
- If using EndA$^+$ host strains, grow in Luria Broth and purify DNA from only 100 ml of culture using recommended volumes of resuspension, lysis, and neutralization solutions and use twice the normal amount of wash buffer noted.
- The cell resuspension solution contains RNase A. There is no need to add an RNase step to the procedure.

2.2 Protocol

All steps are carried out at room temperature unless otherwise indicated. Centrifugation can be carried out at 4°C. The entire procedure should take less than 1.5 hr.

1. Harvest 100–500 ml of cells, depending on culture density (see above), by centrifugation at 5,000 x g for 5 min (in 250–500 ml centrifuge bottles). Discard the supernatant.
2. Add 15 ml of cell resuspension solution to the cell pellet. Vortex the cells or pipet up and down to resuspend the pellet. Be sure that the cells are completely resuspended.
3. Add 23 ml of cell lysis solution. Mix by swirling the centrifuge bottle. Do not vortex, since this may cause shearing of the chromosomal DNA resulting in contamination of the plasmid DNA. The solution should become viscous and somewhat clear after a few minutes. Do not incubate longer than 5 min before proceeding to step 4.
4. Add 15 ml of neutralization solution. Mix by swirling the centrifuge bottle. The solution should become cloudy and develop a flocculant white precipitate. Proceed directly to step 5. Do not let sit longer than 10 min before proceeding to step 5.
5. Centrifuge for 20 min at 8–10,000 x g. Carefully pour the supernatant (cleared lysate) containing the plasmid DNA into a new centrifuge bottle. If the pellet is not well

adhered to the wall of the centrifuge bottle, filter the supernatant through cheesecloth or miracloth. Try not to transfer any of the precipitate; however, a small amount of the floating debris will not affect subsequent purification steps.

6. Resuspend the Quantum Prep matrix by shaking vigorously. Add 10 ml of thoroughly resuspended Quantum Prep matrix to the cleared lysate from step 5. Swirl gently for 15–30 sec to mix. Centrifuge for 5 min at 3,000 x g to pellet the matrix.
7. Pour off the supernatant from the pelleted matrix. **Add 95–100% ethanol to the wash buffer before first use. Ethanol should be added up to the base of the neck of the bottle. Store the bottle capped tightly to preserve the correct ethanol concentration**.
8. Add 25 ml of wash buffer to the pelleted matrix. Resuspend the matrix in the wash buffer by shaking. **If isolating DNA from an EndA$^+$ strain, use 50 ml of wash buffer**.
   Additional maxiprep wash buffer can be made by mixing 200 mM NaCl, 40 mM Tris, 4 mM EDTA, pH 7.5 with an equal volume of 95–100% ethanol.
9. Centrifuge for 3–5 min at 3,000 x g.
10. Insert a spin basket into a 50 ml screwcap centrifuge tube (supplied).
11. Pour off the supernatant from the pelleted matrix. Add 15 ml of wash buffer to the pellet. Resuspend the matrix

and then transfer the suspension to the spin basket from step 10. Do not replace the lid of the tube. Centrifuge the spin basket/tube for 5 min at 4,000 rpm (3,000 x g) in a swinging bucket rotor.

12. Remove the basket and pour out the filtrate from the tube. Replace the basket in the tube and add 10 ml of wash buffer to the matrix in the basket. Centrifuge again for 5 min at 4,000 rpm (3,000 x g).

    Repeat the 15 ml and 10 ml wash steps if using an EndA^{+} strain.

13. Remove the basket to a new sterile 50 ml centrifuge tube (supplied). Add 5 ml of sterile water or TE. Centrifuge for 5 min at 4,000 rpm (3,000 x g). DNA at this stage is ready to use. If additional purity or concentration is desired, the sample may be precipitated with ethanol as described below.
14. Precipitate the DNA by adding 1/18 volume of 5 M NaCl (e.g., 222 µl/4 ml eluate) and 2 volumes (e.g., 8 ml/4 ml eluate) of cold 95–100% ethanol (not supplied).
15. Pellet the DNA by centrifuging at 4,000 x g or greater for 10–20 min.
16. Wash the pellet by adding 10 ml of 70% ethanol, then centrifuging at 4,000 x g or greater for 10 min. Allow the pellet to dry (air dry or vacuum) before resuspending it in an appropriate amount of TE or sterile water. Do not overdry the pellet as this may create difficulty in resuspending the DNA.

Section 3. References

1. U.S. Patent 5,075,430 issued to Bio-Rad Laboratories.
2. Ausubel et al., Current Protocols in Molecular Biology, Wiley-Interscience, New York (1987)

Section 4. Product Information

Catalog #	Description
732-6120	**Quantum Prep Plasmid Midiprep Kit,** 20 preps
732-6100	**Quantum Prep Plasmid Miniprep Kit,** 100 preps
732-6110	**Quantum Prep Matrix,** 20 ml

Catalog #	Description
732-6340	**AquaPure™ Genomic DNA Isolation Kit,** for cultured cells and gram-negative bacteria; processes up to 100 cultured cell preps (1–2 x 10^6 cells/prep) or 100 x 0.5 ml bacterial cell cultures per kit
732-6345	**AquaPure Genomic DNA Blood Kit,** for human and mammalian whole blood and bone marrow; processes up to 100 x 0.3 ml whole blood samples per kit
732-6343	**AquaPure Genomic DNA Tissue Kit,** for animal and plant tissues, cultured cells, and gram-negative bacteria; processes up to 100 (0.5–10 mg) animal and plant tissue preps, 100 cultured cell preps (1–2 x 10^6 cells/prep), or 100 x 0.5 ml bacterial cell cultures per kit

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