
**Quantum Prep®
HT/96 ClearSpin
Plasmid Miniprep Kit**

**Catalog Numbers
732-6151
732-6153**



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Section 1 Introduction to Quantum Prep HT/96 Plasmid Prep Kits

1.1 Overview

The Quantum Prep HT/96 plasmid prep kits utilize the patented DNA binding matrix found in the Quantum Prep plasmid mini, midi and maxi prep kits to yield high throughput preparations of ultrapure plasmid DNA. Vacuum filtration is used to reduce the hands-on time involved.

This protocol has been optimized for the isolation of high-copy number plasmids grown in 1–1.8 ml of culture media. Yields of up to 15 micrograms 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 HT/96 ClearSpin plasmid miniprep kit (catalog number 732-6151) contains materials and reagents sufficient for 2 x 96 plasmid minipreps. Catalog number 732-6153 consists of five of the 2 x 96 kits and therefore provides enough materials and reagents for 10 x 96 plasmid minipreps.

45 ml	Cell Resuspension Solution
45 ml	Cell Lysis Solution
45 ml	Neutralization Solution
22 ml	Quantum Prep Matrix
50 ml	HT/96 Wash Buffer
2	96 well (2 ml) Grow Blocks

2	96 well (800 µl) Matrix Filter Plates
2	96 well (250 µl conical bottom) Collection Plates w/ Lids
2	96 well (1.1 ml) Collection Tubes in Racks
2	Waste Trays (Top of Collection Tubes)
24	8 Strip Caps for Collection Tubes
2	Packs Sealing Tape (4 per pack)

1.3 Storage and Stability

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

Section 2 Protocol

There are no restrictions on the use of rich media such as Terrific Broth or the final O.D.₆₀₀ of the bacterial culture with ClearSpin. Simply take care to avoid the flocculant precipitate when transferring the centrifuge-cleared lysate to the Matrix Filter Plate (see below).

2.1 Recommendations for Best Results

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 protocol has been optimized for the isolation of high-copy number plasmids grown in 1–1.8 ml of culture media.

The use of a host strain which contains a mutation in the endonuclease I gene (*endA*) is highly recommended, such as JM109, DH5α®, DH10, or XL1-Blue. 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.

A precipitate may form in either the lysis or neutralization solutions due to colder ambient temperatures encountered in winter shipping conditions, cool laboratory temperatures, or factory storage conditions. This precipitation will not affect the 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.

- 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 concentration is desired, the sample may be evaporated or alcohol precipitated.
- The use of strains deficient in the endonuclease I gene product (*endA1* genotype), such as DH5αF', is recommended for improving the quality of plasmid DNA prepared from minipreps.
- A centrifuge with rotor carrier adaptor for deep well (2.0 ml) 96 well plates is required to pellet the bacterial cultures before beginning the alkaline lysis prep. If you do not have a centrifuge with these adaptors, you will have to transfer resuspended pellets to the grow block and begin at step 3 below.
- Dry Grow Block with Kimwipe in order to allow a proper seal with seal tape at steps 3 & 4.

- A centrifuge with rotor carrier adapter for deep well (2.0 ml) 96 well plates is also required to clear the bacterial cell lysates for the ClearSpin protocol. If you do not have a centrifuge with these adaptors, you will have to transfer the lysates to microcentrifuge tubes to clear them in a standard microcentrifuge, then transfer the cleared lysates to the Matrix Filter Plate at step 7 below. The ClearVac kit (catalog number 732-6150, 732-6152) allows vacuum filtration of the bacterial cell lysates.
- Although vacuum filtration adds considerable convenience to the prep, centrifugation is more efficient for the removal of residual wash buffer (step 10) as well as for elution of plasmid DNA (step 12). See Options to Protocol below for details.

2.2 Use of the Vacuum Manifold

The Vacuum Manifold, catalog number 732-6170, is for use with the HT/96 Plasmid Miniprep Kits but is not included. However, other commercially available manifolds will work with the HT/96 kits. Vacuum filtration is used to reduce the hands-on time involved in performing plasmid minipreps.

The Vacuum Manifold consists of three parts, the base, the housing and the gaskets. The hose barb is fit by friction into a hole in the side of the housing. Assemble the manifold by first setting one gasket on the base (orange side up), then centering whichever collection device is appropriate (Grow Block or Collection Tube/Rack) onto the base. Place the housing over the collection device, insuring that the top gasket is also in position (orange side up) on top of the housing. Finally, set the appropriate filter plate onto the top of the housing.

Use the 3' Tygon tubing provided (1/2" O.D., 3/8" ID) to attach the hose barb in the housing to your vacuum source. House vacuum is usu-

ally sufficient, 20"–25" Hg is optimal. A regulator may be used but is not supplied.

2.3 Protocol

All steps are carried out at room temperature unless otherwise indicated. Centrifugation can be carried out at 4 °C. Release vacuum slowly so as not to disturb pellets. The entire procedure should take less than 40 minutes.

1. Harvest cells in 2 ml Grow Block at 1,500 x g for 5 min.
2. Add 200 µl Cell Resuspension Solution to each well. Place lid on Grow Block and vortex briefly to resuspend cells. (Lid is packaged with collection plate.)
3. Add 200 µl Lysis Solution to each well. Seal Grow Block with sealing tape. Invert 5 times to mix.
4. Remove tape. Add 200 µl of Neutralization Solution to each well. Seal Grow Block with sealing tape. Invert 5 times to mix.
5. Spin Grow Block at 2,000 x g for 15 minutes to clear the lysate.
6. During the spin, place the top of the Collection Tubes/Rack upside down onto the Vacuum Manifold Base for use as a waste tray for the collection of flow-through and wash in the next few steps. Place the Vacuum Manifold Housing onto the base and place the Matrix Filter Plate on top of the housing.
7. After the spin, remove tape and use a pipette set on 500 µl to transfer the supernatants from the Grow Block to the Matrix Filter Plate. Take care to avoid the flocculant debris.
8. Insure that the Quantum Prep matrix is thoroughly resuspended by shaking vigorously. Add 100 µl of matrix to each well of the Matrix Filter Plate. Pipet up and down 2–3 times to mix lysate and matrix.

Apply vacuum until all of the liquid has passed through the filter (about 1–2 min).

The correct final formulation of the HT/96 Wash Buffer is 80% ethanol, added by the user. To achieve this formulation, add four volumes, 200 ml, of 95% or 100% ethanol to the HT/96 Wash Buffer before use of HT/96 kit.

9. Add 500 µl of Wash Buffer to each well of the Matrix Filter Plate. Apply vacuum and allow Wash Buffer to pass through the matrix (about 1 min).
10. Add an additional 500 µl of Wash Buffer to each well of the Matrix Filter Plate. Seal all wells of the plate with Sealing Tape. Again apply vacuum and allow Wash Buffer to pass through the matrix. Remove residual Wash Buffer by leaving vacuum on for a full 3 min. Release vacuum by slowly removing sealing tape. (Centrifugation is an alternative; see Options to Protocol.)
11. Remove the Matrix Filter Plate from the top of the Vacuum Manifold. Blot the drip directors with a clean Kimwipe to remove any adhering buffer. Lift the housing from the base of the Vacuum Manifold and remove the Waste Tray and its contents.
12. Place the Collection Tubes onto the Vacuum Manifold Base. Place the Vacuum Manifold Housing onto the base. Remove the sealing tape from the Matrix Filter Plate and place the plate on top of the Vacuum Manifold. Pipet 80 µl of ddH₂O or TE into each well to elute DNA. (50–100 µl may also be used; see Helpful Hints below.) Again seal all wells of the plate with sealing tape. Wait 1 min, then apply vacuum for 3 min. (Centrifugation is an alternative; see Options to Protocol.)
13. Remove and discard Matrix Filter Plate. Lift housing and remove collection tubes from the Vacuum Manifold. At this point you may

either cap the collection tubes for storage of the DNA or transfer to another type of container.

2.4 Options to the ClearSpin Protocol

1. After step 3.

You may want to centrifuge the Grow Block for several seconds to remove residual liquid from the top of the wells before removing the sealing tape in order to feel more secure about avoiding cross contamination. It can also insure that the tape will stick firmly at step 4. However, in our experience it is not necessary to do either.

2. At steps 7 and 8.

Instead of pipetting the centrifuge-cleared lysate into the Matrix Filter Plate and then adding 100 µl of matrix, you can instead aliquot 100 µl of Matrix into another Grow Block (not provided), transfer the centrifuge-cleared lysate to it and mix by pumping up and down. This may be useful if for any reason there will be delays in your procedure between transferring the lysate to the Matrix Filter Plate and the addition of the matrix.

When you are ready to filter the matrix, place the Matrix Filter Plate on top of the Vacuum Manifold. Pipet the lysate/matrix mixture up and down twice in the Grow Block and then transfer to the Matrix Filter Plate for filtration at step 8. Be sure to transfer any matrix, which has settled to the bottom of the Grow Block.

3. At step 10.

For more efficient removal of residual Wash Buffer, centrifuge the filter plate for 3 min at 1,500 x g with a collection plate underneath.

4. At step 12.

DNA may also be eluted into the supplied Collection Plate using the Collection Tube Rack as a booster beneath the plate.

5. Elution of DNA may be more efficiently accomplished by centrifuging. If you used the collection plate previously for the wash step above, be sure to rinse and dry it thoroughly before use or use a clean collection plate (catalog number 732-6157). Place a collection plate underneath the Matrix Filter Plate.

Pipet 50–100 μl of ddH₂O, TE, or 10 mM Tris pH 8.0 into each well depending on concentration desired. It is not necessary to seal all wells of the plate with tape if centrifuging to elute. Place Lid on top of Matrix Filter Plate. Wait 1 min then spin at 2,000 x g for 3 min. Use sealing tape for storage in the collection plate or transfer to a container of your choice.

2.5 Helpful Hints

1. Sealing all wells with sealing tape at step 10 (final wash step) and step 12 (elution step) provides significantly greater vacuum pressure in order to move fluid through the filter plate. Release vacuum by slowly removing sealing tape.
2. Check to insure that the drip directors of the Matrix Filter Plate are within the Collection Tubes for elution by vacuum at step 12.
3. The Vacuum Manifold can be used without a Waste Tray. If used in this manner, the base should be rinsed in water before the elution step.
4. To do less than 96 preps, simply use sealing tape to cover the unused wells of the Grow Block and Matrix Filter Plate. Marking with a pen can indicate previously used columns or rows.
5. Increasing the elution volume will decrease the concentration (ng/ μl) of the final product but will increase the total yield in micrograms. Decreasing the elution volume will have the opposite effect. The suggested 50–100 μl elution volume should provide a concentration adequate to move directly to a cycle sequencing reaction without further concentration or manipulation.

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 Number	Product Description
732-6151	Quantum Prep HT/96 ClearSpin Plasmid Miniprep Kit , 2 x 96 preps
732-6153	Quantum Prep HT/96 ClearSpin Plasmid Miniprep Kit , 10 x 96 preps
732-6150	Quantum Prep HT/96 ClearVac Plasmid Miniprep Kit , 2 x 96 preps
732-6152	Quantum Prep HT/96 ClearVac Plasmid Miniprep Kit , 10 x 96 preps
732-6154	800 μl 96 well Lysate Filter Plates , 10
732-6155	800 μl 96 well Matrix Filter Plates , 10
732-6156	2.0 ml 96 well Grow Blocks , 10
732-6157	250 μl 96 well Collection Plates with Lids , 10
732-6158	1.1 ml 96 well Collection Tubes/Rack , 10
732-6159	8 Strip Caps for Collection Tubes , 120
732-6262	Sealing Tape , 20 sheets

Catalog Number	Product Description
732-6170	Vacuum Manifold , 1
732-6171	Vacuum Manifold Gaskets , 2
732-6100	Quantum Prep Plasmid Miniprep Kit , 100 preps
732-6120	Quantum Prep Plasmid Midiprep Kit , 20 preps
732-6130	Quantum Prep Plasmid Maxiprep Kit , 10 preps
732-6110	Quantum Prep Matrix , 20 ml
732-6122	Quantum Prep Neutralization Solution , 110 ml
732-6024	Quantum Prep Wash Buffer , 250 ml

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