

Plasmid MaxiSpin System

**Catalog Number 200-010
10 purification**

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I. Introduction

The Plasmid MaxiSpin System

The MaxiSpin System is a spin cartridge-based plasmid purification system which is designed to produce several hundred micrograms of plasmid or cosmid DNA of exceptional purity. -purified plasmid and cosmid is excellent for even the most demanding molecular biology applications, such as mammalian cell transfection. It provides a fast, low-cost alternative to both cesium chloride gradients and expensive, inconsistent and time-consuming anion-exchange columns. Each preparation consists of a chromatographic spin cartridge and a 50 ml conical centrifuge tube. During the course of the purification, more than one conical tube will be required but the exact number of conical tubes used in each purification will vary depending upon the volume of culture processed and individual preferences. The tube provided with the spin cartridge is nuclease- and endotoxin-free and is intended to be used for recovery of the purified plasmid during the final elution step (Step 13). The customer is expected to provide any 50 ml conical tubes used prior to Steps 12-13.

Column capacity and buffer volumes

The volumetric capacity of each spin cartridge is approximately 20 ml and the plasmid binding capacity is approximately 700 µg. The Maxi Protocol is intended to assimilate LB cultures of 100-200 ml. Each 100 ml of LB overnight culture will generate a clarified lysate volume (Step 6) of approximately 17 ml. Therefore, when starting with 200 ml LB cultures, the clarified lysate (approx. 34 ml total) will be loaded onto the spin cartridge in two sequential steps (Steps 7-8 in protocol) and all of the plasmid consolidated onto the chromatographic membranes. The following outline describes the volumes of each of the buffers added when starting with different size bacterial cultures:

200 ml LB or 40 ml SB/TB/2xYT:

- 10 ml Buffer 1
- 10 ml Buffer 2
- 14 ml Buffer 3
- 20 ml Buffer 4
- 2.2 ml Buffer 5

100 ml LB or 20 ml SB/TB/2xYT:

- 5 ml Buffer 1
- 5 ml Buffer 2
- 7 ml Buffer 3
- 20 ml Buffer 4
- 2.2 ml Buffer 5

Bacterial cultures and plasmid copy numbers

This protocol is intended for users with experience in the growth of bacterial cultures and some understanding of DNA purification methods, DNA handling, plasmid propagation, etc. There are a number of molecular biology laboratory manuals currently available which provide instruction on the formulation of bacterial broths and the propagation of plasmids. Individuals unfamiliar with these techniques should consult such a reference before proceeding.

Always inoculate the primary cultures with a single colony from a fresh plate and never grow for more than 16 hours. 100 and 200 ml cultures are typically inoculated with either an aliquot of the primary culture (e.g., 100-200 µl) or from a single colony and grown to stationary phase. Also, do not store the bacterial cultures for extended periods (particularly at room temperature) before initiating plasmid purification or significant amounts of nicked plasmid may result.

The actual amount of plasmid or cosmid rendered will depend primarily upon the copy number of the construct, culture volume and media composition. High copy number plasmids, such as pGEM and pUC will generally produce approximately 2-5 µg/ml of LB culture. These same high copy number constructs will generate up to approximately 10-20 µg/ml when grown in richer broths, such as SB, TB or 2xYT. Medium and lower copy number plasmids (e.g., pET and pBR322) and cosmids will generate approximately 0.2-0.5 µg/ml of LB culture and approximately 1-2.5 µg/ml of SB, TB or 2xYT. These are only general figures but are sufficiently accurate to allow one to plan the propagation and purification of their constructs accordingly.

Very low- and single-copy plasmids

Very low copy number plasmids (e.g., P1) can also be purified using the procedure but the reagent volumes in the kit have not been configured for the routine purification of these constructs. These low copy number constructs must typically be grown in very large culture volumes in order to produce enough plasmid for the intended application, which would quickly exhaust the kit reagents. Therefore, this kit is not recommended for their purification. Ana-Gen offers larger volumes of its buffers and modified protocols specifically for purification of these large, single-copy constructs. *Please inquire.*

Centrifugation

The following protocol designates 400 x g, 3,000 x g and 20,000 x g at various steps. These centrifugal force figures are provided because they have demonstrated optimal and highly reproducible performance, in terms of spin cartridge hydrodynamics, plasmid yield and purity. However, it has also been our experience that the MaxiSpin cartridge is inherently robust and can be used with a variety of different centrifuges, in a number of different rotors (either fixed angle or swingling bucket) and under a range of different centrifugal forces and achieve plasmid yields and purity which are indistinguishable in quality. Therefore, one should not necessarily be concerned if they must carry out this procedure under conditions which are different than those described. However, the following considerations **MUST** be observed:

- a. In Step 6., sufficient force must be employed to completely clarify the lysate and compact the debris against the bottom of the tube. **Cloudy lysates cannot be loaded into the spin cartridge because occlusion of the chromatographic membranes will result.** Clarification of the lysate will generally require a centrifugal force of 15-20,000 x g for 15 minutes in an appropriate high-speed centrifuge tube.
- b. Centrifugal forces must be achieved which will allow the complete movement of the entire fluid volume through the chromatographic membranes. When using g-forces other than those recommended, be sure to check the spin cartridge after each centrifugation for residual liquid resting on top of the chromatographic membranes. Spin longer and/or at a higher speed if necessary.
- c. Try to avoid subjecting the spin cartridge to g-forces >4,000 x g. Excessive g-force may distort the chromatographic membranes and alter the cartridge hydrodynamics.

Each purification is configured to process up to 200 ml of LB culture or 40 ml of SB/TB/2xYT culture and up to approximately 700 µg of plasmid DNA. When smaller amounts of plasmid or cosmid are required, 100 ml LB or 20 ml SB/TB/2xYT bacterial cultures can also be processed by simply reducing the volumes of Buffers 1-3 by one-half.

II. Kit Components

Plasmid MaxiSpin Buffers

Only Buffer 1 should be stored at 4°C. All other buffers and kit components should be stored at room temperature. The minimum shelf-life of Buffer 1 is nine months when stored at 4°C. All other buffers have a minimum shelf-life of at least nine months when stored at room temperature. The buffers used in this kit are NOT interchangeable with those in the Plasmid Miniprep Kit.

Each kit of 10 purifications contains the following buffer volumes:

Plasmid MaxiSpin Buffer 1 (105 ml)

- Proprietary composition
- Contains RNase A
- Nontoxic and noncaustic
- Recommended storage at 4°C if kit is consumed over several months.

Plasmid MaxiSpin Buffer 2 (105 ml)

- Proprietary composition
- Caustic: contains NaOH
- Always wear eye protection and gloves when handling

Plasmid MaxiSpin Buffer 3 (150 ml)

- Proprietary composition
- Irritant: contains a mix of salts, acetic acid and nontoxic additives
- Always wear eye protection and gloves when handling

Plasmid MaxiSpin Buffer 4 (45 ml 5x)

- 10 mM Tris-Cl, pH 7.5 (final)
- 80% ethanol (final)

IMPORTANT: The customer is required to add 180 ml of 95-100% ethanol to the contents of the Buffer 4 bottle to generate the working wash buffer. Either denatured or nondenatured ethanol may be used.

Plasmid MaxiSpin Buffer 5 (40 ml)

- 12 mM Tris-Cl, pH 8.5
- Nontoxic and noncaustic

Spin cartridges and collection tubes

- 10 MaxiSpin chromatographic cartridges
- 10 - 50 ml conical centrifuge tubes

III. Supplied by the Customer

Plasticware

- Additional 50 ml conical centrifuge tubes
- 50 ml high-speed (Oak Ridge) centrifuge tubes

Equipment required

- Centrifuge and rotor capable of centrifuging 50 ml conical tubes at a maximum of 5,000 rpm (approximately 3,000 x g). Either swinging bucket or fixed angle rotors are acceptable.
- Centrifuge and rotor capable of centrifuging 50 ml high-speed centrifuge tubes at 20,000 x g.

IV. Protocol

Read all steps carefully before proceeding.

1. Pellet the bacteria by centrifugation for 10 minutes at 3,000 x g*, 4 °C.

Bacterial cultures grown to stationary phase. Select one of the following:

- 100 ml of high- or medium copy number construct in LB
- 200 ml of high- or medium copy number construct in LB
- 20 ml of either high- or medium copy number construct in SB, TB or 2xYT
- 40 ml of either high- or medium copy number construct in SB, TB or 2xYT

Aliquot the bacterial culture into an appropriate number of 50 ml conical (Falcon-type) tubes or centrifuge bottle. Alternatively, 50 ml "Oak Ridge" high-speed centrifuge tubes can also be used.

Note: Alkaline lysis of the bacterial pellet (Step 4) is intended to occur in a 50 ml high-speed centrifuge tube. Therefore, either the bacterial pellet or the resuspended bacteria (Step 3) must be in a high-speed centrifuge tube prior to the addition of Buffer 2.

IMPORTANT: DO NOT EXCEED THE RECOMMENDED CULTURE VOLUMES, ABOVE.

*3,000 x g is approximately 5,000 rpm when using a Beckman C0650 fixed angle or comparable rotor. Be sure to check specifications for the particular rotor used.

2. After centrifugation, carefully pour off the supernatant, invert the tubes and remove any residual broth by pressing the end against a paper towel. After centrifugation, inspect the broth for clarity to verify complete pelleting of the bacteria. The pellets should be firm and securely impacted against the wall of the tube.

3. Resuspend the bacterial pellet derived from each 200 ml of LB culture or 40 ml of SB/TB/2xYT in a total of 10 ml of Plasmid Maxi Buffer 1.

Add 10 ml of Buffer 1 and either pipet or vortex (moderate speed) the bacterial pellet until it is completely resuspended.

For 100 ml of LB culture or 20 ml of SB/TB/2xYT:

Resuspend the pellet in a total of 5 ml of Buffer 1.

Note: If the bacterial culture was pelleted in more than one tube (aliquot), divide the Buffer 1 accordingly. For example, if the 200 ml culture was divided into four 50 ml tubes for centrifugation, add 2.5 ml of Buffer 1 to each tube. Consolidate before the addition of Buffer 2.

Note: If the resuspended bacterial pellet is in a low-speed conical tube, it must be transferred to a high-speed centrifuge tube at this time. To avoid shearing of the genomic DNA, no transfer should occur after the addition of Buffer 2.

4. Add a total of 10 ml of Plasmid Maxi Buffer 2 to the resuspended bacteria derived from 200 ml of LB culture or 40 ml of SB/TB/2xYT.

Following the addition of Buffer 2, the contents should be mixed by gently inverting 8-10 times.

For 100 ml of LB culture or 20 ml of SB/TB/2xYT:

Add a total of 5 ml of Buffer 2.

Caution: This step lyses the bacteria and releases both the plasmid and the bacterial chromosomal DNA into solution. The release of the bacterial DNA results in a noticeable increase in the viscosity of the solution. The bacterial DNA is very brittle and will fracture into smaller pieces if agitated excessively. Small pieces of bacterial chromosomal DNA will copurify with the plasmid if present. To avoid this, do not agitate the solution forcefully and **NEVER VORTEX**.

Note: Following the addition of Buffer 2, neutralization with Buffer 3 (Step 5) should occur within 5 minutes.

5. Add a total of 14 ml of Plasmid Maxi Buffer 3 to the bacterial lysate derived from 200 ml of LB culture or 40 ml of SB/TB/2xYT and gently invert several (8-10) times.

This step renders the bacterial chromosomal DNA and cell wall debris insoluble through a combination of rapid neutralization in conjunction with a high salt-detergent precipitation. The plasmid is left in solution. Following the addition of Buffer 3 and mixing, a white debris precipitate will immediately form.

Important: Do not vortex or agitate with excessive force but be sure that the contents are homogeneously mixed during the inversions.

For 100 ml of LB culture or 20 ml of SB/TB/2xYT:

Add a total of 7 ml of Buffer 3.

6. Centrifuge for 15 minutes at a minimum of 20,000 x g* in a high-speed centrifuge.

This step will tightly pellet the precipitate and suspended material against the bottom or wall of the tube allowing easy removal of the clarified supernatant containing the plasmid.

Note: After centrifugation, inspect the pellet to be sure that it is tightly adhering to the wall of the tube. If the pellet appears loose or diffuse, increase the speed setting by 2,000 rpm and centrifuge for an additional 10 minutes. Be sure to increase the centrifugation speed accordingly during the next preparation.

Note: After centrifugation, the lysate should be completely clear. Cloudiness indicates that an insufficient time and/or g-force was/were applied. **DO NOT TRANSFER CLOUDY LYSATES TO THE SPIN CARTRIDGE.** If the lysate appears cloudy, increase the speed setting by 2,000 rpm and centrifuge again for 10 minutes. Be sure to increase the centrifugation speed accordingly during the next preparation.

Note: Plasmid Buffers 1-3 have been specifically formulated to produce firm, immobile genomic DNA pellets following precipitation and centrifugation. However, these reagents can be overwhelmed if too many bacteria are processed. If the white pellet appears gelatinous and diffuse following centrifugation, it is an indication that too many cells have been processed and an excessive amount of bacterial chromosomal DNA is present. This gelatinous mass can be removed by partially drawing it into the pipet tip and transferring it out of the tube. Following this, centrifuge again at high speed for 10 minutes. This will not affect the quality of the plasmid preparation. Reduce the culture volume by 20-25% in subsequent preparations.

Note: At the end of this centrifugation you will have either ≤ 17 ml of clarified lysate or ≤ 34 ml of clarified lysate. In the next step, the 17 ml aliquot of lysate is loaded onto the spin cartridge in a single step. The 34 ml lysate is loaded in two separate steps (ie., Steps 7 & 8 are repeated).

*20,000 x g is approximately 15,000 rpm when using a Beckman F0850 fixed angle or comparable rotor. Be sure to check specifications for the particular rotor used.

7. Pour the clarified supernatant into a MaxiSpin cartridge in a 50 ml conical tube.

Carefully pour off the supernatant without disturbing the pellet. A small amount of particulate matter transferred to the spin column is inconsequential but plasmid quality will be optimized if this step is carried out with finesse. Steps 7 & 8 will be repeated if starting with 200 ml of LB culture.

8. Bind the plasmid to the spin cartridge membrane with a low speed spin.

Centrifuge the spin column for **5 minutes at 400 x g***. The binding of the plasmid to the spin cartridge membrane is dependent upon a diffusive interaction between the plasmid and the surface of the fibers comprising the membrane. This interaction is facilitated by moving the plasmid-bearing solution through the membrane at a modest speed.

Note: After centrifugation, inspect the spin cartridge to be sure that all of the lysate has flowed through the chromatographic membranes. If any lysate remains above the membranes, spin for an additional 5 minutes.

*400 x g is approximately 2,000 rpm when using a Beckman C0650 fixed angle or comparable rotor. Be sure to check specifications for the particular rotor used.

If starting with 200 ml of LB culture or 40 ml of SB/TB/2xYT:

Repeat Steps 7 & 8 with the second 17 ml aliquot of clarified lysate. Each spin cartridge can accommodate the lysate derived from up to 200 ml of LB culture or up to 40 ml of SB/TB/2xYT.

9. Wash the spin cartridge with 20 ml of Plasmid Maxi Buffer 4.

Add 20 ml of Buffer 4 to the spin cartridge and centrifuge for **5 minutes at 400 x g**.

Note: After centrifugation, inspect the spin cartridge to be sure that all of the Buffer 4 has flowed through the chromatographic membranes. If any remains above the membranes, spin for an additional 5 minutes.

10. Empty the wash buffer from the collection tube and centrifuge the spin cartridge for 10 minutes at 3,000 x g. This step is necessary to completely purge the chromatographic membranes of residual wash buffer. If possible, remove the conical tube cap during this spin.

11. Transfer the spin cartridge to the 50 ml conical tube it was packaged with or other fresh 50 ml conical tube.

Check to ensure that no solution remains around the exterior of the spin cartridge. If necessary, dry the exterior with a clean tissue (e.g., kimwipe).

Note: Alternatively, any clean, sterile 50 ml conical tube should be satisfactory for this step.

12. Elute the bound DNA with 2.2 ml of Plasmid Maxi Buffer 5.

Pipet 2.2 ml (standard eluent volume) into the spin cartridge and **allow the elution buffer to be absorbed by the chromatographic membranes for 5 minutes before proceeding**. Then **spin for 10 minutes at 3,000 x g**.

Note: TE or deionized water can also be used as eluents, provided that the pH is 8.0-8.5. However, the chemical stability of DNA is diminished when stored in pure (nonbuffered) water for extended periods. Some investigators have reported the inhibition of enzymatic reactions, such as PCR, when even small

amounts (0.5-1 mM) of EDTA are present. Speed-vac concentration of plasmids eluted in TE may concentrate the EDTA to a level which may interfere with subsequent enzymatic reactions.

Note: After centrifugation, check the volume of recovered eluent. If the volume is not ≥ 2.0 ml, spin for an additional 5 minutes at the maximum speed possible. If the volume of the recovered eluent is > 2.2 ml, it indicates that residual wash buffer remained in the spin cartridge in Step 11. The eluent will probably also have an ethanol odor. Follow the steps below for precipitating the plasmid.

Ethanol precipitation (optional)

On occasion, there may be a trace of ethanol or salt remaining in the purified plasmid solution. In any application where a small amount of these reagents may be deleterious, we recommend precipitating the plasmid according to the procedure below. **For mammalian cell transfection we recommend routinely precipitating the construct of interest prior to transfection.**

Note: Following elution, high copy number constructs will generally exhibit a concentration of approximately ≥ 200 ng/ μ l of eluent. If this concentration is not sufficient for the intended application, please follow the precipitation procedure, below.

- Add 1/3 volume 7.5 M ammonium acetate
- Add 2 volumes 95-100% ethanol
- Chill for 1 hour at -80°C or dry ice, or overnight at -20°C
- Centrifuge for 30 minutes at $\geq 15,000 \times g$ in a 4°C high-speed centrifuge
- Wash the pellet 2x with ice-cold 70% ethanol
- Air-dry the pellet and resuspend in an appropriate volume of deionized water, 10 mM Tris-Cl or TE.

V. Troubleshooting

1. Poor yield

The most likely causes are as follows:

- A medium- or lower copy number construct is being purified from an excessively large culture volume and the RNase A activity of Buffer 1 has been overwhelmed. In this scenario the much more abundant RNA is swamping the spin column membrane and excluding the plasmid from binding. This would be confirmed by the presence of a large amount of RNA when evaluating the preparation on a gel.
- Instead of adding 95-100% ethanol to the Buffer 5 concentrate, a lower concentration (e.g., 70%) was added instead. In this scenario, the plasmid is eluting prematurely in the wash buffer.
- The bacterial culture is heterogenous and contains a low titer of plasmid-bearing bacteria.
- The plasmid is simply present in a lower number of copies per cell than anticipated.

Remedial measures:

- Reduce the culture volume.
- Restreak the bacterial culture and grow another culture from a single colony.
- Replace the Buffer 1.

2. RNA contamination

RNA contamination is visualized as a diffuse low molecular weight band or smear at the bottom of each lane on an agarose gel. The most common causes of residual RNA contamination are as follows:

- Processing excessive amounts of bacteria (the RNase A activity in Buffer 1 is overwhelmed).
- The RNase A activity in Buffer 1 has been compromised due to either age or improper storage.
- The bacterial pellet was incompletely resuspended in Buffer 1 prior to the addition of Buffer 2.

Remedial measures:

- Reduce the bacterial culture volume by 20-25%.
- Replace the Plasmid Buffer 1.
- Vortex or pipet until the cell pellet is completely resuspended. Visually inspect the cell suspension to ensure that complete resuspension has occurred.

3. Genomic DNA contamination

Residual bacterial genomic DNA is depicted as a high molecular weight band in or near the well on an agarose gel. The most common causes of genomic DNA contamination in a plasmid preparation are as follows:

- Processing excessive amounts of bacteria.
- Insufficient centrifugation in Step 6.
- Incomplete (nonhomogenous) mixing of Plasmid Buffers 2 and 3.
- Excessive force when mixing in Buffers 2 and 3.

Remedial measures:

- Run a preparative agarose gel and gel-purify the plasmid to remove the contaminating DNA.
- Increase the speed of centrifugation in Step 6.
- Repeat the purification and reduce the culture volume by 20-25%.
- Following the addition of Buffers 2 and 3 use gentle but thorough mixing to ensure that the contents are completely mixed without shearing the genomic DNA.

4. When run on a gel, the plasmid looks “dirty” with high background or other debris.

This is generally accompanied by a reduced $A_{260/280}$. Plasmid purified with the method will generally exhibit an $A_{260/280}$ of 1.8-1.9. An $A_{260/280} < 1.7$ indicates that proteinaceous debris may have been carried over with the plasmid during purification. High background on an ethidium bromide-stained agarose gel is a further indication that there was inefficient segregation of the plasmid from other bacterial components during the purification regimen. The most common causes are as follows:

- Processing inordinately large bacterial culture volumes.
- Incomplete mixing of Buffers 1-3 with the bacterial pellet.

- Incorrect volume(s) of Buffer(s) 1-3.
- Insufficient centrifugation during step 6.

Remedial measures:

- Reduce the bacterial culture volume.
- Reacquaint oneself with the protocol to ensure that correct volumes of buffers are used and complete mixing occurs at all steps.
- Pay particular attention to the appearance of the pellet after centrifugation during Step 6. Also, the supernatant generated in Step 6 must be completely clear before loading into the spin cartridge.

5. Poor performance in enzymatic reactions or transfections.

Generally this indicates the presence of a contaminant which was incompletely removed from the plasmid during the purification process. There are several contaminants which may affect enzymatic reactions and/or transfections adversely:

- Bacterial genomic DNA
- Bacterial metabolites and cell wall constituents (ie., carbohydrates, lipids and proteins)
- Chaotropic salts
- Ethanol

Remedial measures:

- Ethanol precipitate to salvage plasmid from current preparation.
- Reduce the bacterial culture volume in subsequent preparations.
- If possible, try another bacterial host for plasmid propagation.
- Chill the bacterial lysate on ice for 20 minutes after the addition of Buffer 3.
- Routinely ethanol precipitate the recovered plasmid after elution in Step 13.

For Technical Assistance, please contact Ana-Gen at (404)223-5090.

VI. Product Warranty

Ana-Gen Technologies (Ana-Gen) products are sold for research purposes only. Each production lot is subjected to appropriate quality assurance procedures and are warranted to perform as indicated when handled, stored and used according to the manufacturer's instructions. Please report any problems to Ana-Gen immediately by calling (404)223-5090. By using this product, the customer acknowledges and agrees that any liability which Ana-Gen incurs to the customer is at the discretion of Ana-Gen and is limited to either the replacement of the product or in certain cases, to a credit of the price paid to Ana-Gen for the product, including applicable shipping and handling costs. The customer further agrees that any liability assumed by, or any remedial action taken by Ana-Gen is contingent upon the customer providing full details of the nature of the problem and the circumstances of use to Ana-Gen, including return of the product to Ana-Gen for testing. Any remedial measures taken by Ana-Gen will occur only AFTER testing and verification of malperformance by Ana-Gen of the product in question. **Ana-Gen will not, under any circumstances provide the specific composition of solutions, reagents or any kit component which it regards and/or has designated as "proprietary".**

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