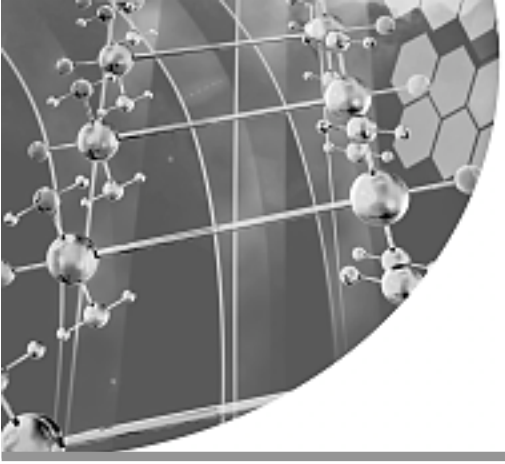

Manual FastPlasmid™ Mini Kit

For rapid plasmid purification in just
9 minutes

Contents

Introduction

The FastPlasmid™ Mini Kit is based on a proprietary technology that provides a rapid, nonorganic method for isolating high purity plasmid DNA from 1.5 to 3 ml of Escherichia coli bacterial cultures. This innovative technology has dramatically transformed traditional alkaline lysis by eliminating multiple solutions and time-consuming steps associated with the conventional plasmid purification process.

The FastPlasmid™ Mini Kit technology is different from conventional alkaline lysis procedures. It uses a single solution for cell resuspension, lysis, and DNA trapping. This single solution contains a mixture of enzymes and detergents that lyse bacterial cells, denatures and solubilizes cellular components, degrades RNA, and traps DNA on a matrix—all in one step. The solution is initially chilled to 0°C, then added to the bacterial pellet and vortexed at high speed for thirty seconds. This mixing step not only resuspends the pellet but also allows cellular debris to be degraded by the enzymes and detergents. The resuspended cell mixture is incubated for three minutes at room temperature and transferred to a mini spin column. This incubation is critical for removing a wide range of proteins by changing the solubility, preventing co-purification with the DNA. The DNA is washed with an alcohol- based solution to remove any remaining impurities. Finally, a low-salt elution buffer is added to the matrix to dissolve the DNA, which is collected in a fresh tube using a short centrifugation step. This entire process takes less than half the time of the other purification kits and yields pure DNA suitable for the following downstream applications:

- Restriction digestion
- Cloning
- Ligation
- PCR
- Sequencing
- Transformation
- In vitro transcription

Expected Yields with the FastPlasmid™ Mini Kit

Yields for high-copy and low-copy vectors will vary depending on the *E. coli* host. For high-copy vectors, yields up to 20 µg for a 1.5 ml overnight culture are possible. Yields for low-copy vectors range from approximately 1.5 µg to 3 µg for a 1.5 ml overnight culture.

Precautions and warnings

Appropriate safety apparel such as lab coat, gloves, and eye protection should be worn. For more information, please consult the appropriate material safety data sheets which are available for this kit online at www.5 PRIME.com/msds.

Materials

Materials supplied in the kits

Component	100 Preps	250 Preps
Lysis Solution	56 ml	140 ml
RNase Solution	0.7 ml	1.75 ml
Lysozyme	60 mg	150 mg
Wash Buffer Concentrate	18 ml	45 ml
Elution Buffer (10 mM Tris-Cl, 0.1 mM EDTA, pH 8.5)	10 ml	25 ml
Culture Tube	100 each	250 each
FastPlasmid™ Spin Column Assembly (Spin Column and Waste Tube)	100 each	250 each

Materials required but not supplied

- Liquid bacterial growth medium supplemented with the appropriate antibiotic (see Section 7.1)
- Isopropanol (95 –100%)
- Microcentrifuge minimally capable of achieving spin speeds of 12,000 x g / 13,000 rpm

Storage and stability

All FastPlasmid™ Mini Kit components are stable for at least 12 months when stored unopened at room temperature.

Important information: Upon arrival, remove the RNase A and the Lysozyme from the kit box and store them at 2-8°C.

It is recommended that the Lysis Solution be stored at 4°C in preparation for use.

The Complete Lysis Solution (Lysis Solution plus the RNase/Lysozyme mix) must be stored at 4°C and is stable for 4 months when stored unopened.
The Complete Lysis Solution is stable for up to 6 months if it is aliquoted into smaller volumes using sterile technique and then stored at 4°C to prevent contamination.

Store all other FastPlasmid™ Mini Kit components at room temperature. Do not freeze.

Store DILUTED Wash Buffer at room temperature in a tightly sealed container to prevent evaporation of the isopropanol.

Quality assurance

Each lot of FastPlasmid™ Mini Kit is tested by use in the isolation of plasmid DNA as described in the protocol. The plasmid DNA is evaluated for quantity and quality by spectrophotometric assay, agarose gel electrophoresis, complete digestion with restriction endonucleases, and automated fluorescent sequencing.

Protocol

Procedures described in this section have been optimized for the special requirements of various hosts and/or vectors. Please read the following information specific to each procedure and choose the procedure that best fits your needs.

The **detailed protocol** (page 9) may be used with most host/vector combinations and provides sufficient yield for downstream applications.

- For *end*A+ bacterial strains see page 10 on removing endonucleases when using *end*A+ bacterial strains such as HB101, JM110 and DH12S.
- For low-copy and expression vectors see page 10 on processing low-copy and expression vectors such as pGEX and pET.

Before starting

- FastPlasmid™ is very different from traditional plasmid purification kits. First time users should familiarize themselves with the entire protocol before starting.

Growth of bacterial cultures

Maximum plasmid DNA yields are obtained when optimal growth conditions are employed. These conditions are achieved by using a single isolated colony from a freshly transformed or freshly plated E. coli bacterial strain, and inoculating in culture medium.

Glucose should not be added to the culture medium. The addition of glucose to the culture medium will significantly reduce plasmid yield. Other sugars may also have the same effect as glucose and should not be used to supplement the growth medium.

Growth in LB culture medium is strongly recommended. Growth in nutrient-rich media, such as 2x YT and TB, is not recommended as this can produce significantly higher cell densities and overload the purification system.

If using a small amount of a frozen glycerol stock as inoculum, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. After overnight incubation, a well-separated colony should be picked and used to inoculate the culture medium. Significant decreases in plasmid DNA yield have been observed when certain plasmid-bearing strains are continuously propagated in the laboratory. This also may occur when the inoculum for a new culture is taken from a previously grown culture or an old agar plate. Although this procedure may be acceptable for many host/vector combinations, it does not ensure optimal plasmid yields in every case.

→ When using the FastPlasmid™ Mini Kit, incubate culture to an OD_{600} of 2.0 to 4.0 (12 to 16 hours at 37°C with sufficient aeration). Dilute the sample to obtain an OD_{600} in the linear range of the instrument.

IMPORTANT PROCESSING STEPS

Prepare the Complete Lysis Solution (Lysis Solution plus the RNase/Lysozyme mix):

To ensure optimum lysis conditions, store the Lysis Solution, RNase Solution, and the lyophilized Lysozyme at 4°C prior to use.

1. Briefly centrifuge the RNase Solution to collect all of the liquid in the bottom of the tube. Resuspend the lyophilized Lysozyme using the entire volume of the RNase Solution. Do not use the tube containing the Lysozyme as a centrifuge balance tube as this may cause difficulty in resuspending the Lysozyme.
2. Mix thoroughly by pipetting up and down. Take care to ensure that all of the powder is dissolved. Some foaming will occur.
3. Pipet the entire contents of the resuspended Lysozyme/RNase mixture to the Lysis Solution bottle. This will be referred to as Complete Lysis Solution. Mix the bottle thoroughly and check the "Enzyme Mix Added" box on the label. Store the Complete Lysis Solution at 4°C.
   → It may be necessary to rinse the Lysozyme tube with a small volume of Lysis Solution in order to collect the entire volume of the RNase/Lysozyme mixture.

ICE-COLD (0–4°C) Complete Lysis Solution is required for optimum kit performance.

Chill the Complete Lysis Solution on ice prior to use. The Complete Lysis Solution can be incubated on ice for an indefinite period of time without affecting the performance of the kit.

→ Note: If the Complete Lysis Solution has NOT been stored at 4°C prior to use, chill on ice until the solution is at 4°C or less. This may take as long as 45 minutes if the Lysis Solution was room temperature before starting.

Prepare DILUTED Wash Buffer:

→ Add the appropriate volume of isopropanol (95 –100%) to the entire bottle of Wash Buffer Concentrate as indicated in the table below. Mix the bottle thoroughly.

Kit size	Volume of isopropanol
100 prep	38 ml
250 prep	95 ml

Detailed protocol

1. Chill the Complete Lysis Solution on ice.
   → If the Complete Lysis Solution has NOT been stored at 4°C prior to use, chill on ice until the solution is at 4°C or less (may require up to 45 minutes).
2. Pellet 1.5 ml of fresh bacterial culture at maximum speed (at least 12,000 x g or 13,000 rpm) for 1 minute in the provided 2 ml Culture Tube.
   → See page 4 before using the kit for the first time for suggestions on the growth of bacterial cultures.
3. Remove medium by decanting, taking care not to disturb bacterial pellet. Inverting the tubes on a paper towel may improve removal of the medium.
   → If necessary, a maximum of 3 ml of culture can be processed.
4. **Add 400 µl of ICE-COLD Complete Lysis Solution.**
   → The Complete Lysis Solution MUST be ice-cold (0–4°C) to obtain maximum yield.
5. **Mix thoroughly by constant vortexing at the highest setting for a full 30 seconds.** This step is critical for obtaining maximum yield.
   → If the pellet is not completely resuspended, continue vortexing until the lysate is a homogenous solution with no apparent cell clumps visible. Several tubes may be processed at the same time using a Thermomixer (see page 11) or vortexing using a foam water bath tube rack.
6. Incubate the lysate at room temperature for 3 minutes.
   → The lysate should appear non-viscous and slightly cloudy, with no precipitate.
7. Transfer the lysate to a Spin Column Assembly by decanting or pipetting.
8. Centrifuge the Spin Column Assembly for 30–60 seconds at maximum speed.
   → It is not necessary to decant filtrate after Step 8. Wash Buffer may be added directly to the Spin Column Assembly in the centrifuge.
9. Add 400 µl of DILUTED Wash Buffer to the Spin Column Assembly.
10. Centrifuge the Spin Column Assembly for 30–60 seconds at maximum speed.
11. Remove the Spin Column from the centrifuge and decant the filtrate from the Waste Tube. Place the Spin Column back into the Waste Tube and return it to the centrifuge.
12. Centrifuge at maximum speed for 1 minute to dry the Spin Column.
13. Transfer the Spin Column to a Collection Tube.

14. Add 50 µl of Elution Buffer directly to the center of the Spin Column membrane and cap the Collection Tube over the Spin Column.
    - ➔ To avoid inconsistent elution volumes, ensure that the elution buffer is pipetted directly onto the surface of the filter, avoiding contact with the wall of the column.
15. Centrifuge at maximum speed for 30– 60 seconds.
16. Remove and discard the Spin Column.
17. The eluted DNA can be used immediately for downstream applications or stored at -20°C.

Additional protocol information

_end_A⁺ bacterial strains

This procedure is recommended for removing endonucleases that are present when using *end*A+ strains such as HB101, JM110 and DH12S. The endonucleases are removed by using a Guanidine Hydrochloride/ Isopropanol wash after trapping the DNA on the matrix in Step 8 of the detailed protocol.

- ➔ Before starting, prepare 100 ml of a 1 M Guanidine Hydrochloride/50% Isopropanol Solution.
  a. Dissolve 9.55 grams of Guanidine HCl in 40 ml of Molecular Biology Grade Water.
  b. Bring the volume to 50 ml with Molecular Biology Grade Water.
  c. Mix the Guanidine HCl solution with 50 ml of Isopropanol (95 – 100%).
- ➔ Following Step 8 in the detailed protocol, add 400 µl of the 1 M Guanidine HCl / 50% Isopropanol Solution to the Spin Column Assembly.
- ➔ Centrifuge the Spin Column Assembly for 30 – 60 seconds at maximum speed.
- ➔ Remove the Spin Column from the centrifuge and decant the filtrate from the Spin Column Assembly Waste Tube. Place the Spin Column back into the Waste Tube and return it to the centrifuge.
- ➔ Proceed with Step 9 of the detailed protocol.

Low-copy and expression vectors

This procedure is recommended for low-copy and expression vectors such as pGEX and pET. This step is incorporated at the DNA elution step of the detailed protocol. The addition of this step has been shown to increase yields when using these vectors.

- ➔ Following the addition of the Elution Buffer in Step 14 of the protocol, incubate the sample for 5 minutes.
- ➔ Proceed with Step 15 of the detailed protocol.

Thermomixer

This procedure is recommended when processing several tubes at the same time and will take the place of Steps 5 and 6 of the detailed protocol. A Thermomixer with a 1.5 or 2.0 ml block may be used.

- Attach the block to the Thermomixer and verify that the Thermomixer is at room temperature (22 – 25°C) for this incubation.
- Following Step 4 of the detailed protocol, place culture tubes onto the Thermomixer and mix at 1,400 rpm for 3 minutes.
- Proceed with Step 7 of the detailed protocol.

Troubleshooting

Problem	Possible cause	Resolution
Low quality DNA	The Lysis Solution may not be cold enough.	Ensure the Lysis Solution is between 0°C and 4°C.
	The bacterial pellet may not have been fully resuspended. Proper mixing of the Lysis Solution and the bacterial pellet is critical for removal of cellular contaminants.	Vortex bacterial pellet for at least 30 seconds. Longer resuspension times may be required in some cases.
	Inflated absorbance readings have periodically been observed with various bacterial strains.	Ensure that the Lysis Solution is between 0°C and 4°C and that the bacterial pellet has been completely resuspended. If necessary, increase incubation times (Step 6).
	The Wash Buffer was diluted incorrectly.	Make sure that 95% –100% isopropanol was added to the Wash Buffer Concentrate bottle according to directions.

Troubleshooting

Problem	Possible cause	Resolution
Low DNA yield	The plasmid did not propagate.	Make sure that the appropriate antibiotic was included during all stages of growth.
	The cell resuspension was incomplete.	Vortex for at least 30 seconds. Check for a homogenous solution with no apparent cell clumps.
	The lysate was not incubated long enough (Step 6).	Make sure that the lysate is incubated for at least 3 minutes. Longer incubations (up to 5 minutes) may increase yield.
	The Wash Buffer Concentrate was diluted incorrectly.	Make sure that 95% –100% isopropanol was added to the Wash Buffer Concentrate bottle according to directions.
	Problematic hosts and/or vectors (e.g. XL1Blue).	Yields may be increased with the addition of isopropanol to the lysate. Add 125 µl of isopropanol (95 –100%) to the lysate after the incubation in Step 6 and then transfer the lysate to the Spin Column Assembly and proceed with the protocol.
Filter membrane clogs during elution	The bacterial culture may have been grown outside of the recommended OD_{600} range.	Additional eluate may be recovered by increasing spin times (Step 15). Decrease the volume of bacterial culture used for plasmid DNA preparation.

Additional information

Average plasmid concentrations with FastPlasmid™ Mini Kit

The following tables show the average plasmid concentrations and total recoveries of four different high-copy plasmids propagated in three *E.coli* hosts. The plasmid DNAs were purified from 1.5 ml LB cultures using the detailed protocol.

	XL1Blue		TOP10	
Plasmid	**Concentration (ng/µl)**	**Recovery (µg)**	**Concentration (ng/µl)**	**Recovery (µg)**
pUC19	115	5.8	162	8.1
pBluescript II	179	9.0	224	11.2
pSV-β-gal	182	9.1	421	21.1
pEGFP-n1	74	3.7	106	5.3

	DH10B	
Plasmid	**Concentration (ng/µl)**	**Recovery (µg)**
pUC19	110	5.5
pBluescript II	186	9.3
pSV-β-gal	231	11.6
pEGFP-n1	96	4.8

The following tables show the average plasmid concentrations and total recoveries of three different low-copy plasmids, propagated in two E. coli hosts. Plasmids were purified from 1.5 ml LB cultures using the FastPlasmid™ Mini Kit kit using the protocol on page 9.

	DH10B	DH10B	HB101	HB101
Plasmid	**Concentration (ng/µl)**	**Recovery (µg)**	**Concentration (ng/µl)**	**Recovery (µg)**
pACYC	51	2.6	39	1.9
pBR322	62	3.3	50	2.9
pET19	32	1.6	28	1.4

Host strains

Several strains of *E coli* have proven themselves as reliable propagators of plasmids. The most common strains are DH5α, DH10B, and Top10.

JM101 and JM109 bacterial pellets may be difficult to resuspend. Additional vortexing may be required to ensure complete resuspension.

Ordering information

Product	Package Size	Catalog No.
FastPlasmid™ Mini Kit	100 Preps	2300000
FastPlasmid™ Mini Kit	250 Preps	2300010
Perfectprep® Plasmid 96 Vac Kit	2 Plates	2300200
Perfectprep® Plasmid 96 Vac Kit	10 Plates	2300210
Perfectprep® Plasmid 96 Vac Base Kit	50 Plates	2300220
Collection Plates	50 Plates	2300230
Culture Plates - 50	50 Plates	2300240
Perfectprep® BAC 96 Kit	2 Plates	2300300
Perfectprep® BAC 96 Kit	10 Plates	2300310
Perfectprep® BAC 96 Base Kit	50 Plates	2300320
Water, Mol Bio grade	1 l	2500000
Water, Mol Bio grade	10 x 50 ml	2500010
Water, Mol Bio grade	5 l	2500020
DNA Gel Loading Buffer 10x	6 x 500 µl	2500070
TBE 5x	5 l	2500050
TAE 50x	5 l	2500060
Phase Lock Gel™ Light 1.5 ml	200 Tubes	2302800
Phase Lock Gel™ Light 2 ml	200 Tubes	2302820
Phase Lock Gel™ Light 15 ml	100 Tubes	2302840
Phase Lock Gel™ Light 50 ml	25 Tubes	2302860
Phase Lock Gel™ Heavy 1.5 ml	200 Tubes	2302810
Phase Lock Gel™ Heavy 2 ml	200 Tubes	2302830
Phase Lock Gel™ Heavy 15 ml	100 Tubes	2302850
Phase Lock Gel™ Heavy 50 ml	25 Tubes	2302870

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Standard protocol summary

1. Chill the Complete Lysis Solution on ice.
2. Pellet 1.5 ml of fresh bacterial culture at maximum speed (at least 12,000 x g or 13,000 rpm) for 1 minute in the provided 2 ml Culture Tube.
   → See page 6 before using the kit for the first time for suggestions on the growth of bacterial cultures.
3. Remove medium by decanting, taking care not to disturb bacterial pellet.
4. **Add 400 µl of ICE-COLD Complete Lysis Solution.**
   → The Complete Lysis Solution MUST be ice-cold (0 –4°C) to obtain maximum yield.
5. **Mix thoroughly by constant vortexing at the highest setting for a full 30 seconds.** This step is critical for obtaining maximum yield.
6. Incubate the lysate at room temperature for 3 minutes.
7. Transfer the lysate to a Spin Column Assembly by decanting or pipetting.
8. Centrifuge the Spin Column Assembly for 30– 60 seconds at maximum speed.
9. Add 400 µl of DILUTED Wash Buffer to the Spin Column Assembly.
10. Centrifuge the Spin Column Assembly for 30–60 seconds at maximum speed.
11. Remove the Spin Column from the centrifuge and decant the filtrate from the Waste Tube. Place the Spin Column back into the Waste Tube and return it to the centrifuge.
12. Centrifuge at maximum speed for 1 minute to dry the Spin Column.
13. Transfer the Spin Column into a Collection Tube.
14. Add 50 µl of Elution Buffer directly to the center of the Spin Column membrane and cap the the Collection Tube over the Spin Column.
15. Centrifuge at maximum speed for 30 – 60 seconds.
16. Remove and discard the Spin Column.
17. The eluted DNA can be used immediately for downstream applications or stored at -20°C.

FastPlasmid™ Mini Kit quick protocol

1.5 ml Bacterial Culture: Centrifuge for 1 minute to pellet the cells

Decant the media

Add 400 µl of ICE-COLD Complete Lysis Solution
Vortex for a full 30 seconds at the highest setting

Incubate at room temperature for 3 minutes

Transfer the lysate to a Spin Column Assembly

Centrifuge for 30 – 60 seconds

Wash DNA with 400 µl of DILUTED Wash Buffer

Centrifuge for 30 – 60 seconds

Decant the filtrate from the waste tube and re-assemble the Spin Column Assembly

Centrifuge for 1 minute

Transfer the Spin Column into a Collection Tube

Add 50 µl of Elution Buffer to the center of the Spin Column

Centrifuge for 30 – 60 seconds

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