ISOLATE II
Plasmid Mini Kit

Product Manual

BIOLINE

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ISOLATE II Plasmid Mini Kit

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1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps	250 Preps
ISOLATE II Plasmid Mini Spin Columns (white)	10	50	250
Collection Tubes (2ml)	10	50	250
Resuspension Buffer P1	5ml	15ml	75ml
Lysis Buffer P2	5ml	15ml	3 x 25ml
Neutralization Buffer P3	5ml	20ml	100ml
Wash Buffer PW1	6ml	30ml	2 x 75ml
Wash Buffer PW2† (concentrate)	2ml	2 x 6ml	2 x 20ml
Elution Buffer P	5ml	15ml	75ml
RNase A (lyophilized)	2mg	6mg	30mg
Product Manual	1	1	1
Bench Protocol Sheet	1	1	1

† Before use, add indicated volume of 96-100% ethanol and mark wash buffer bottle label.

2. DESCRIPTION

The ISOLATE II Plasmid Mini Kit is a simple, reliable and fast method for isolation of high-quality plasmid DNA from *E. coli* host cells by SDS/alkaline lysis. The lysate is subsequently neutralized and adjusted to high-salt binding conditions in one step. After lysate clearing, the sample is ready for purification on a silica membrane to which the plasmid binds. Any contamination and impurities such as salts, metabolites and cellular components are effectively removed by simple washing steps with two different buffers. High-quality purified plasmid is then eluted in an elution buffer.

Please read this manual carefully to familiarize yourself with the ISOLATE II Plasmid Mini protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

3. STORAGE

Store Resuspension Buffer P1 containing RNase A at 4°C (stable for at least 6 months). All other kit components should be stored at room temperature (18–25°C) and are stable for up to 1 year. Storage at lower temperatures may cause precipitation of salts. If a precipitate of the SDS is observed in Lysis Buffer P2, incubate the bottle at 30–40°C for several minutes and mix well. Always keep buffer bottles tightly closed, especially if buffers are pre-heated during the preparation.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Neutralization Buffer P3 and Wash Buffer PW1 contain guanidine hydrochloride. This chemical is harmful when in skin contact, inhaled or ingested.

For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

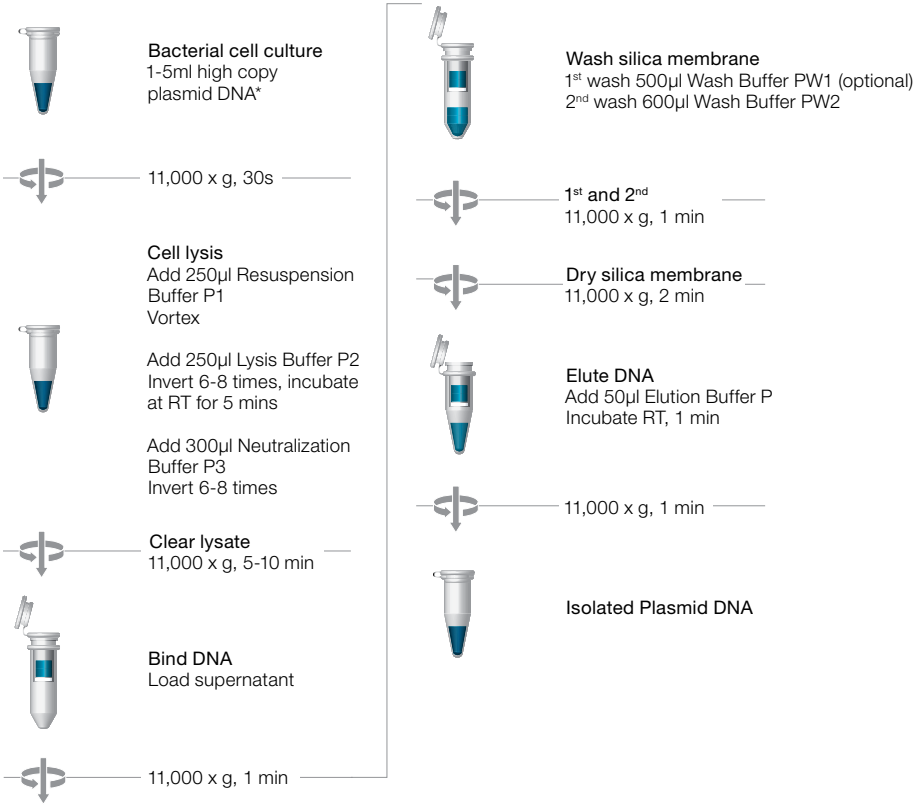
5. PRODUCT SPECIFICATIONS

The ISOLATE II Plasmid Mini Kit is specially designed for the small-scale rapid and efficient isolation of extremely pure plasmid DNA. The Plasmid Mini Columns offers very high DNA binding capacity of up to 60µg, provided there is thorough washing, which is strongly recommended for host strains with high levels of endonucleases like HB101 or JM110.

The ISOLATE II Plasmid Mini Kit allows purification of low-copy plasmids from larger culture volumes, purification of plasmids from Gram-positive bacteria and clean-up of plasmids from reaction mixtures. The purified plasmid DNA is suitable for applications such as fluorescent DNA sequencing, PCR and enzymatic manipulation.

ISOLATE II PLASMID MINI COLUMN SPECIFICATIONS	
Max. binding capacity	60µg plasmid DNA
Culture volume	1-5ml high copy 5-10ml low copy
Typical yield	<25µg (1-5ml culture) <40µg (5-10ml culture)
Elution volume	50µl
Max. plasmid size	<15kb
Hands on preparation time	25 min /18 preps

Plasmid DNA Isolation



** For low copy plasmids, use 5-10ml bacterial culture and double volume of buffers P1, P2 and P3 for lysis.*

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves.

- 96-100% ethanol†
- Microcentrifuge tubes (1.5ml)
- Sterile DNase-free tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)
- Vortex mixer
- Thermal heating block

† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone.

7. IMPORTANT NOTES

7.1 GROWTH OF BACTERIAL CULTURES

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic (Table 1). The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic and type of culture medium.

TABLE 1: ANTIBIOTICS

Antibiotic*	Stock solution (concentration)	Storage	Working concentration
Ampicillin	100mg/ml in water	-20°C	50-200µg/ml
Carbenicillin	100mg/ml in ethanol	-20°C	20-200µg/ml
Chloramphenicol	50mg/ml in ethanol	-20°C	25-170µg/ml
Kanamycin	10mg/ml in water	-20°C	10-50µg/ml
Neomycin	50mg/ml in water	-20°C	50µg/ml
Tetracycline	12.5mg/ml in ethanol	-20°C	12.5-50µg/ml

** See associated products*

Plasmids vary widely in their copy number per cell (Table 2), depending on their origin of replication (e.g. ColE1, pMB1 or pSC101) which determines whether they are under relaxed or stringent control. Also, depending on the size of the plasmid and its associated insert, overall yield can be affected.

TABLE 2: ORIGINS OF REPLICATION AND COPY NUMBERS			
Plasmid type*	Origin of replication	Copy number	Classification
pTZ	pMB1	>1000	High copy
pUC	pMB1	500–700	High copy
pBluescript	ColE1	300–500	High copy
pGEM®	pMB1	300–400	High copy
pBR322 and derivatives	pMB1	15–20	Low copy
pACYC and derivatives	p15A	10–12	Low copy
pSC101 and derivatives	pSC101	~5	Very low copy

** See associated products*

For cultivation of bacterial cells harbouring standard high-copy plasmids, we recommend Lysogeny Broth (LB) medium. Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. The cell culture should be incubated at 37°C with constant shaking (200-250 rpm) overnight (12-16 h), to give an OD of 3-6. Alternatively, rich media like 2 x YT (Yeast/Tryptone) or TB (Terrific Broth) can be used. In this case bacteria grow faster, reach the stationary phase much sooner (≤12 h) with a higher cell mass. However, this does not necessarily yield more plasmid DNA.

Growth for more than 16 hours (12 hours for rich media) is not recommended since cells begin to lyse and plasmid yields may be reduced. This can also lead to contamination with chromosomal DNA. To find the optimal culture conditions, the culture medium and incubation times should be optimized for each host strain / plasmid construct combination individually. As a general guide we recommend using 5ml of a well grown culture (for more accurate guide see Table 3).

TABLE 3: RECOMMENDED CULTURE VOLUMES ACCORDING TO THE OPTICAL DENSITY						
OD_{600}	1	2	3	4	5	6
Culture volume	15ml	8ml	5ml	4ml	3ml	2ml

Note: If excess culture volume is used, alkaline lysis will be inefficient, the membrane will be overloaded and performance will decrease. If more than the recommended amount of cells shall be processed, refer to the support protocol for low-copy plasmid purification (section 9.1).

7.2 BUFFER PREPARATION AND PARAMETERS

Preparing Resuspension Buffer P1

Add 1ml of Resuspension Buffer P1 to the RNase A vial and vortex. Transfer all of the resulting solution into the Resuspension Buffer P1 bottle and mix thoroughly. Store Resuspension Buffer P1 containing RNase A at 4°C.

Note: The solution will be stable at this temperature for at least six months.

Preparing Wash Buffer PW2

Add 96–100% ethanol to Wash Buffer PW2 Concentrate: 8ml for the 10 prep kit, 24ml x 2 for the 50 prep kit and 80ml x 2 for the 250 prep kit.

Alternative Elution Parameters

It is possible to modify the standard elution protocol to improve yield and concentration.

Use Elution Buffer P preheated to 70°C for one of the following procedures:

- High yield, especially for larger constructs: Heat Elution Buffer P to 70°C, add 50-100µl to the column and incubate at 70°C for 2 min.
- High yield: Two elution steps with 50µl Elution Buffer P (to increase yield to 90–100%).
- High concentration: One elution step with 30µl Elution Buffer P (to increase concentration by about 130%). Maximal yield 80%.
- High yield and high concentration: Two elution steps. Add 25µl Elution Buffer P, incubate for 3 min and centrifuge, repeat with a second 25µl Elution Buffer P. Yield 85–100% at a high concentration.

8. PROTOCOL

8.1 ISOLATION OF HIGH-COPY PLASMID DNA FROM *E. COLI*

Before you start:

- Make sure Wash Buffer PW2 and Resuspension Buffer P1 are prepared (see section 7.2).

1 **Harvest bacterial cells**

Use 1-5ml of a saturated *E. coli* LB culture (see section 7.1), pellet cells for 30s at 11,000 x g. Discard the supernatant and remove as much liquid as possible.

Note: For isolation of low-copy plasmids refer to section 9.1.

2 **Lyse cells**

- Add 250µl Resuspension Buffer P1 and resuspend the cell pellet completely by vortexing or pipetting up and down, making sure no cell clumps remain.
- Add 250µl Lysis Buffer P2. Mix gently by inverting the tube 6-8 times.

Note: Do not vortex to avoid shearing of genomic DNA.

Incubate at room temperature for up to 5 min or until lysate appears clear.

- Add 300µl Neutralization Buffer P3. Mix thoroughly by inverting the tube 6-8 times.

Note: Do not vortex to avoid shearing of genomic DNA.

3 **Clarification of lysate**

Centrifuge for 5 min at 11,000 x g at room temperature.

Repeat this step if supernatant is not clear.

4 **Bind DNA**

For each preparation, take one ISOLATE II Plasmid Mini Spin Column, placed in a Collection Tube and decant or pipette a maximum of 750µl of the clarified sample supernatant onto the column. Centrifuge for 1 min at 11,000 x g and discard flow-through.

Repeat with any remaining clarified sample supernatant.

5 **Wash silica membrane**

If plasmid DNA is prepared from host strains containing high levels of nucleases (e.g. HB101 or strains of the JM series), we strongly recommend performing an additional wash step at this point with Wash Buffer PW1.

(Optional) Add 500µl Wash Buffer PW1 preheated to 50°C and centrifuge for 1 min at 11,000 x g before proceeding.

Note: Additional washing with Wash Buffer PW1 will also increase the read length of DNA sequencing reactions and improve the performance of critical enzymatic reactions.

Add 600µl Wash Buffer PW2 (supplemented with ethanol) and centrifuge 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.

6 **Dry silica membrane**

Centrifuge 2 min at 11,000 x g, to remove residual ethanol. Place the ISOLATE II Plasmid Mini Spin Column in a 1.5ml microcentrifuge tube (not supplied).

7 **Elute DNA**

Add 50µl Elution Buffer P directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Note: For alternative elution procedures see section 7.2.

9. ALTERNATIVE PROTOCOLS

9.1 ISOLATION OF LOW-COPY PLASMID, P1 CONSTRUCTS OR COSMID DNA FROM *E. COLI*

Processing of larger culture volumes requires increased lysis buffer volumes. The buffer volumes provided with the kit are calculated for high-copy plasmid purification only. Therefore, if this protocol is to be used frequently, an additional buffer set can be ordered separately (see ordering information).

Before you start:

- Make sure Wash Buffer PW2 and Resuspension Buffer P1 are prepared (see section 7.2).

1 **Harvest bacterial cells**

Use 5-10ml of a saturated *E. coli* LB culture, pellet cells for 30s at 11,000 x g. Discard the supernatant and remove as much liquid as possible.

2 **Lyse cells**

- Add 500µl Resuspension Buffer P1 and resuspend the cell pellet completely by vortexing or pipetting up and down, making sure no cell clumps remain.
- Add 500µl Lysis Buffer P2. Mix gently by inverting the tube 6-8 times.

Note: Do not vortex to avoid shearing of genomic DNA.

Incubate at room temperature for up to 5 min or until lysate appears clear.

- Add 600µl Neutralization Buffer P3. Mix thoroughly by inverting the tube 6-8 times.

Note: Do not vortex to avoid shearing of genomic DNA.

3 **Clarification of lysate**

Centrifuge for 10 min at 11,000 x g at room temperature.

4 **Bind DNA**

For each preparation, place one ISOLATE II Plasmid Mini Spin Column, in a Collection Tube and decant or pipette 750µl of the clarified sample supernatant onto the column. Ensure all lysate is loaded.

Centrifuge for 1 min at 11,000 x g and discard flow-through.

Repeat with any remaining clarified sample supernatant.

5 **Wash silica membrane**

(Optional) Add 500µl Wash Buffer PW1 preheated to 50°C and centrifuge for 1 min at 11,000 x g before proceeding.

Note: Additional washing with Wash Buffer PW1 will also increase the reading length of DNA sequencing reactions and improve the performance of critical enzymatic reactions.

Add 600µl Wash Buffer PW2 (supplemented with ethanol) and centrifuge 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.

6 **Dry silica membrane**
Centrifuge 2 min at 11,000 x g to remove residual ethanol. Place the ISOLATE II Plasmid Mini Spin Column in a 1.5ml microcentrifuge tube (not supplied).

7 **Elute DNA**
Add 50µl Buffer P preheated to 70°C directly onto the silica membrane. Incubate for 2 min at 70°C. Centrifuge 1 min at 11,000 x g.
Note: For alternative elution procedures see section 7.2.

9.2 ISOLATION OF PLASMID DNA FROM GRAM-POSITIVE BACTERIA

For plasmid purification from bacteria such as *Bacillus* or *Staphylococcus*, it is necessary to start the lysis procedure with an enzymatic treatment (e.g. lysozyme, lysostaphin, mutanolysin) to break up the peptidoglycan layers. For some Gram-positive bacteria such as *Bifidobacteria* or *Corynebacteria*, a preincubation with lysozyme might be insufficient and mechanical cell disruption methods have to be used.

Before you start:
- Make sure Buffer PW2 and Resuspension Buffer P1 are prepared (see section 7.2).

1 **Harvest bacterial cells**
Use 1-5ml of a saturated *E. coli* LB culture, pellet cells for 30s at 11,000 x g. Discard the supernatant and remove as much liquid as possible.

2 **Lyse cells**
- Add 250µl Resuspension Buffer P1 containing 10mg/ml lysozyme (not supplied) and resuspend the cell pellet completely by vortexing or pipetting up and down, making sure no cell clumps remain.

Incubate at 37°C for 10-30 min.
- Add 250µl Lysis Buffer P2. Mix gently by inverting the tube 6-8 times.

Note: Do not vortex to avoid shearing genomic DNA.
Incubate at room temperature for up to 5 min or until lysate appears clear.
- Add 300µl Neutralization Buffer P3. Mix thoroughly by inverting the tube 6-8 times.

Note: Do not vortex to avoid shearing genomic DNA.
Proceed with step 3 of the standard protocol (see section 8.1).

10. TROUBLESHOOTING GUIDE

INCOMPLETE LYSIS OF BACTERIAL CELLS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Cell pellet not properly resuspended	It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of Lysis Buffer P2.
Too many bacterial cells used	We recommend LB as optimal growth medium. If very rich media like TB (Terrific Broth) is used, the cell density of the cultures may become too high and culture time should be reduced.
SDS in Lysis Buffer P2 precipitated	Sodium dodecyl sulfate (SDS) in Lysis Buffer P2 may precipitate if stored at temperatures below 20°C, redissolve by incubating the bottle at 30-40°C for several minutes and mixing well.
POOR PLASMID YIELD	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incomplete lysis of bacterial cells	Cell pellet not properly resuspended, too many bacterial cells used or SDS in Lysis Buffer P2 precipitated, see above.
Suboptimal precipitation of SDS and cell debris	Precipitation of SDS and cell debris will be slightly more effective when centrifuging at 4°C rather than room temperature.
No or insufficient amounts of antibiotic used during cultivation	Add appropriate amounts of freshly prepared stock solutions to all media; both solid and liquid.
Bacterial culture too old	Do not incubate cultures for >16 h at 37°C when using LB and <12 h when using very rich media like TB (Terrific Broth).
Suboptimal elution conditions	If not using Elution Buffer P, make sure elution buffer used is slightly alkaline (pH 8.5). If nuclease-free water is used, check the pH of the water.
No high copy-number plasmid was used	If using low copy-number plasmids, the culture volumes should be increased to at least 5ml.
NO PLASMID YIELD	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Reagents not applied properly	Add indicated volume of 96-100% ethanol to Wash Buffer PW2 Concentrate and mix thoroughly (see section 7.2).
Inappropriate storage of plasmid DNA	Store plasmid DNA dissolved in water at <-18°C or at 4°C when dissolved in Elution Buffer P or TE buffer.
Nuclease-rich host strains used	If using nuclease-rich strains like *E. coli* HB101 or strains of the JM series, keep plasmid preparations on ice or frozen in order to avoid DNA degradation and perform the optional PW1 wash step (step 5; section 8.1). Optimal endonuclease removal can be achieved by incubating the membrane with preheated Wash Buffer PW1 (50°C) for 2 min before centrifugation.

POOR PLASMID QUALITY	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Nicked plasmid DNA due to excessive incubation with Lysis Buffer P2	Cell suspension was incubated with alkaline Lysis Buffer P2 for too long (more than 5 min), incubate for a shorter time.
Genomic DNA contamination	Cell lysate was vortexed or mixed too vigorously after addition of Lysis Buffer P2, shearing the genomic DNA.
Smeared plasmid bands on agarose gel	If using nuclease-rich strains like *E. coli* HB101 or strains of the JM series, keep plasmid preparations on ice or frozen in order to avoid DNA degradation and perform the optional PW1 washing step (step 5; section 8.1). Optimal endonuclease removal can be achieved by incubating the membrane with preheated Wash Buffer PW1 (50°C) for 2 min before centrifugation.
SUBOPTIMAL PERFORMANCE OF PLASMID DNA IN ENZYMATIC REACTIONS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Carry-over of ethanol	Make sure to centrifuge ≥1 min at 11,000 x g in step 6 to achieve total removal of Wash Buffer PW2.
Elution of plasmid DNA with TE buffer	EDTA may inhibit sequencing reactions. Repurify plasmid DNA and elute with Elution Buffer P or water. Alternatively precipitate with ethanol and redissolve in Elution Buffer P or water.
No additional washing with Wash Buffer PW1 performed	Additional washing with 500µl Wash Buffer PW1 (step 5; section 8.1) will increase the reading length of sequencing reactions and improve the performance of critical enzymatic reactions.
Not enough DNA used for sequencing reaction	Quantitate DNA on an agarose gel before setting up sequencing reactions.
Plasmid DNA prepared from too much bacterial cell material	Do not use more than 3ml of a saturated *E. coli* culture if preparing plasmid DNA for DNA sequencing.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@bioline.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Plasmid Mini Kit	10 Preps	BIO-52055
ISOLATE II Plasmid Mini Kit	50 Preps	BIO-52056
ISOLATE II Plasmid Mini Kit	250 Preps	BIO-52057
ISOLATE II Plasmid Buffer Set	1	BIO-52078

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
α-Select Gold Efficiency	1ml (20 x 50µl)	BIO-85027
Agarose	100g	BIO-41026
Ampicillin	10ml (100mg/ml)	BIO-87025
Carbenicillin	10ml (100mg/ml)	BIO-87026
Chloramphenicol	10ml (50mg/ml)	BIO-87027
Kanamycin	10ml (100mg/ml)	BIO-87028
Neomycin	10ml (50mg/ml)	BIO-87029
Tetracycline	10ml (12.5mg/ml)	BIO-87030
IPTG Solution	10ml	BIO-37082
X-Gal	1g	BIO-37035
Quick-Stick Ligase	100 Reactions (10u/µl)	BIO-27028
SOC Medium	10 x 10ml	BIO-86033

D. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace free of charge any product that does not conform to the specifications. This warranty limits Bioline's liability only to the replacement of the product.

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