

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

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### 1 HARVEST BACTERIAL CELLS

Pellet 1-5 mL of a saturated *E. coli* LB culture for 30s at 11,000 x g.  
Discard supernatant and remove as much liquid as possible.

### 2 LYSE CELLS

- Add 250 µL Resuspension Buffer P1 and resuspend cell pellet by vortexing or pipetting up and down.
- Add 250 µL Lysis Buffer P2.  
Mix gently by inverting tube 6-8 times.  
Incubate at room temperature for up to 5 min or until lysate appears clear.
- Add 300 µL Neutralization Buffer P3.  
Mix thoroughly by inverting tube 6-8 times.

### 3 CLARIFICATION OF LYSATE

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

### 4 BIND DNA

Place ISOLATE II Plasmid Mini Spin Column in a 2 mL Collection Tube (supplied).  
Decant or pipette a maximum of 750 µL of clarified sample supernatant onto column.  
Centrifuge 1 min at 11,000 x g and discard flow-through.

### 5 WASH SILICA MEMBRANE

If plasmid DNA is prepared from host strains containing high levels of nucleases, an extra wash with Wash Buffer PW1 is strongly recommended.

(Optional) Add 500 µL Wash Buffer PW1 preheated to 50°C.

Centrifuge 1 min at 11,000 x g.

Add 600 µL Wash Buffer PW2 (supplemented with ethanol).

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 ISOLATE II Plasmid Mini Spin Column in a 1.5 mL microcentrifuge tube (not supplied).

### 7 ELUTE DNA

Add 50 µL Elution Buffer P directly onto center of silica membrane.

Incubate at room temperature for 1 min.

Centrifuge 1 min at 11,000 x g.

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

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If this protocol is to be used frequently, an additional P1, P2 and P3 ISOLATE II Plasmid Buffer Set can be ordered separately.

### 1 HARVEST BACTERIAL CELLS

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

### 2 LYSE CELLS

- Add 500  $\mu$ L Resuspension Buffer P1 and resuspend cell pellet by vortexing or pipetting up and down.
- Add 500  $\mu$ L Lysis Buffer P2.  
Mix gently by inverting tube 6-8 times.  
Incubate at room temperature for up to 5 min or until lysate appears clear.
- Add 600  $\mu$ L Neutralization Buffer P3.  
Mix thoroughly by inverting tube 6-8 times.

### 3 CLARIFICATION OF LYSATE

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

### 4 BIND DNA

Place ISOLATE II Plasmid Mini Spin Column in a 2 mL Collection Tube (supplied).  
Decant or pipette 750  $\mu$ L of clarified sample supernatant onto column.  
Centrifuge 1 min at 11,000 x g and discard flow-through.  
Repeat with any remaining clarified sample supernatant.

### 5 WASH SILICA MEMBRANE

An extra wash with Wash Buffer PW1 is recommended to enhance the performance of downstream applications.  
(Optional) Add 500  $\mu$ L Wash Buffer PW1 preheated to 50°C.  
Centrifuge 1 min at 11,000 x g.  
Add 600  $\mu$ L Wash Buffer PW2 (supplemented with ethanol).  
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 ISOLATE II Plasmid Mini Spin Column in a 1.5 mL microcentrifuge tube (not supplied).

### 7 ELUTE DNA

Add 50  $\mu$ L Buffer P preheated to 70°C directly onto center of silica membrane.  
Incubate for 2 min at 70°C.  
Centrifuge 1 min at 11,000 x g.