

# Plasmid Mini-Prep Kit

## Isolation of Plasmid DNA by silica-gel membrane adsorption

Cat.-No.	Amount
PP-203S	50 preparations
PP-203L	250 preparations

For *in vitro* use only

Quality guaranteed for 12 months

Store at room temperature, RNase A containing Resuspension Buffer should be stored at 4°C

### Kit contents

RNase A

Resuspension Buffer (before use add RNase A)

Lysis Buffer

Neutralization Buffer

Washing Buffer (before use add 96-99% Ethanol)

Elution Buffer

Spin Columns

2 ml Collection Tubes

### To be provided by you

96-99% Ethanol

1.5 ml microtubes

### Description

Plasmid Mini-Prep Kit is designed for isolation of high-purity plasmid or cosmid DNA from cells for subsequent sequencing, restriction digests, or transformations. Spin column based preparation provides an easy and efficient way of DNA isolation without shearing or significant loss of product and allows elution in a small volume of low-salt buffer. It eliminates time-consuming phenol-chloroform extraction or alcohol precipitation and can be used either in micro-centrifuges or on vacuum manifolds. The kit allows the extraction of up to 20 µg DNA per preparation.

### Preparation procedure

The DNA purification follows a simple binding, washing, and eluting procedure. Before start, add 96-99% Ethanol (not included in the kit) to the Washing Buffer as indicated on the bottle. Add the RNase A to the Resuspension Buffer and mix well. RNase A containing Resuspension Buffer should be stored at 4°C. The optional primary washing step minimizes the salt content of the purification product.

#### 1. Cell Harvest and Suspension

- Harvest the cell culture (1 to 3 ml) by centrifugation
- Add 200 µl of Resuspension Buffer (containing RNase A) and resuspend the cell pellet

#### 2. Cell Lysis and Neutralization

- Add 200 µl of Lysis Buffer and mix gently by inverting the tube 4-6 times (Do not vortex!)
- Add 300 µl of Neutralization Buffer and invert the tube immediately 4-6 times
- Centrifuge at 16,000 g for 10 min at room temperature in a microcentrifuge

#### 3. Column Loading

- Place a spin column into a 2 ml collection tube.
- Apply the supernatant from step 2 into the spin column
- Centrifuge at 10,000 g for 30 sec. Discard the flow-through

#### 4. Primary Washing

- Apply 600 µl of Washing Buffer to the spin column
- Centrifuge at 10,000 g for 30 sec and discard the flow-through

#### 5. Column Washing

- Add 400 µl of Washing Buffer to the spin column
- Centrifuge at 10,000 g for 2 min and discard collection tube

#### 6. Elution

- Place the spin column into a clean 1.5 ml microtube (not provided in the kit)
- Add 50 µl Elution Buffer to the center of the column membrane and incubate for 2 min at room temperature
- Centrifuge at 10,000 g for 30 sec to elute DNA