

Plasmid Mini-Prep Kit

Isolation of Plasmid DNA by silica-gel membrane adsorption

Plasmid DNA Purification

	Cat.-No.	Amount
	PP-203XS	10 preparations
	PP-203S	50 preparations
	PP-203L	250 preparations

For *in vitro* use only

Quality guaranteed for 12 months

Store at room temperature, for long term storage

place RNase A lyophilisate at -20°C

RNase A containing Resuspension Buffer should be stored at 4°C

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.

Kit contents

RNase A

Resuspension Buffer (before use, add RNase A)

Lysis Buffer

Neutralization Buffer

Activation Buffer

Washing Buffer (before use, add 96-99% Ethanol as indicated on the bottle)

Elution Buffer

Spin Columns and 2 ml Collection Tubes

To be provided by you

96-99% Ethanol

1.5 ml microtubes

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Plasmid DNA Purification

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:

Cat.-No.	Amount	Ethanol to be added	Final volume
PP-201XS	10 preps	12 ml	15 ml
PP-201S	50 preps	60 ml	75 ml
PP-201L	250 preps	300 ml	375 ml

Add the RNase A to the Resuspension Buffer and mix well. RNase A containing Resuspension Buffer should be stored at 4°C. The optional secondary washing step minimizes the salt content of the purification product.

1 Cell Harvest and Suspension

- Harvest the bacterial cell culture (1-3 ml) by centrifugation
- Resuspend pelleted bacterial cells in 250 µl *Resuspension Buffer* (containing RNase A)

2 Cell Lysis and Neutralization

- Add 250 µl of *Lysis Buffer* and mix gently by inverting the tube 4-6 times (Do not vortex!)
- Add 350 µl of *Neutralization Buffer* and invert the tube immediately 4-6 times
- Centrifuge at 15,000 g for 10 min at room temperature in a microcentrifuge

3 Column Activation

- Place a *Spin Column* into a 2 ml collection tube
- Add 100 µl of *Activation Buffer* into the *Spin Column*
- Centrifuge at 10,000 g for 30 sec in a microcentrifuge

4 Column Loading

- Apply the supernatant from step 2 into the *activated Spin column* by decanting or pipetting
- Centrifuge at 10,000 g for 30 sec
- Discard the flow-through

5 Column Washing

- Place the DNA loaded *Spin Column* into the used 2 ml tube.
- Apply 750 µl of *Washing Buffer* to the *Spin Column*.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.

Optional Secondary Washing: Recommended only for DNA >200 bp, if highly purified DNA (for DNA sequencing, transfection etc.) is required.

- Add 750 µl of *Washing Buffer* to the *Spin Column*.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.
- Centrifuge again for 2 min to remove residual *Washing Buffer*.

6 Elution

- Place the *Spin Column* into a clean 1.5 ml microtube (not provided in the kit)
- Add 30-50 µl *Elution Buffer* or dd-water to the center of the column membrane
- Incubate for 1 min at room temperature
- Centrifuge at 10,000 g for 1 min to elute DNA