mi-Plasmid Midi/Maxiprep Kit

Cat. No. mi-PMD25 & mi-PMD50
Cat. No. mi-PMX10 & mi-PMX25

Troubleshooting Guide

Problem	Possible Reason	Solution
Poor bacterial growth	**Inoculated bacterial sample from a plate or a culture stock stored over a long time period**	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s).
	Inadequate shaking during incubation	Grow cells with vigorous shaking (e.g. 250 rpm). Adjust a suitable shaking speed according to the angular magnitude of an orbital shaker platform.
Poor cell lysis	**Use of excessive amount of bacterial cells harvested from a large or over-grown culture**	Up to 50 (Midi)/100(Maxi) ml culture for high-copy plasmid. Up to 250 (Midi)/500 (Maxi) ml culture for low-copy plasmid. When the culture volume is more than 50(Midi)/100 (Maxi) ml, use increased amount of VP1, VP2 and VP3 Buffer.
	Insufficient resuspension of cell	Do **not** add VP2 Buffer until cells are completely resuspended by vortexing or pipetting.
Low yield of plasmid DNA	**Insufficient amount of bacterial cells**	Ensure that bacteria have been grown well (OD_{600}>1) after overnight incubation at vigorous shaking.
	Overgrowth of bacteria	Incubate bacterial culture with LB medium and do **not** incubate for more than 16 hours.
	Plasmid does not propagate	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s).

Troubleshooting Guide

Problem	Possible Reason	Solution
Low yield of plasmid DNA	**Inefficient or incomplete DNA elution**	Use **no less than 5 ml** VPE Buffer for elution.
	Poor cell lysis	Refer to Solution section of problem – "Poor cell lysis".
	Plasmid is larger than 50-kbp	Use VPE Buffer preheated to 40°C.
Plasmid appears smeared or degraded	**Host strain is *end*A$^+$**	Use *end*A$^-$ strain.
	Overgrowth of bacteria	Incubate bacterial culture with LB medium and do **not** incubate for more than 16 hours.
Genomic DNA contamination in eluate	**Lysate improperly prepared**	After addition of VP2 Buffer, mix gently to prevent shearing of genomic DNA, and do **not** incubate for more than 5 minutes.
RNA contamination	**Insufficient RNase A activity in VP1 Buffer**	Ensure that entire RNase A is added into VP1 Buffer and stored at 4°C. After long-term storage (> 6 months), add RNase A into VP1 Buffer to a conc. of 100µg/ml and store at 4°C.
Plasmid of poor quality	**Eluted DNA contains excessive salt residuals.**	After isopropanol precipitation wash DNA pellet twice with 70% ethanol at room temperature.
	Use of excessive amount of bacterial cells harvested from a large or over-grown culture	Reduce the amount of the used sample. Incubate bacterial culture with LB medium and do not incubate for more than 16 hours.