

mRNex® Endo-free Plasmid Maxi Kit

Cat. no. LM103001

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Product Information

Product	mRNex® Endo-free Plasmid Maxi Kit
Catalog No.	LM103001
Size	10T
Storage conditions	Part I: Components transported with ice bags, stored at -20°C. Part II: Components transported with ice bags, stored at 4°C. Part III: Components transported at room temperature, stored at ambient temperature. Product shelf life: 12 months.

2. Product description

Endo-free Plasmid Maxi Kit is designed for the rapid extraction of 0.5-2 mg of pure, high-copy plasmid DNA from 150-300 mL of Escherichia coli LB culture, with a yield of up to 80-90%. It features high efficiency, speed, and convenience. The silica-based material used in the centrifuge adsorption column is a proprietary, novel material developed by our company. Combined with our unique lysis buffer formula, it allows for the maximum recovery of high-purity DNA. The extracted DNA has high purity, with very low endotoxin contamination (< 0.1 EU/μg DNA), leading to excellent cell transfection results. It can also be directly used in various molecular biology experiments such as restriction digestion, transformation, PCR, in vitro transcription, sequencing, and more.

3. Product component

Form	Cat No.	Component	LM103001(10T)
Part I	LM103001-A	RNaseA (10 mg/mL)	750 μL
	LM103001-B	ER Solution E1	25 mL
Part II	LM103001-C	RS Buffer E1*	77 mL
Part III	LM103001-D	DNA Column E1	10 pieces
	LM103001-E	50 mL Collection Tube E1	20 pieces
	LM103001-F	LB Buffer E1	77 mL
	LM103001-G	BD Buffer E1	77 mL
	LM103001-H	PL Buffer E1*	63 mL
	LM103001-I	Wash Buffer*	50 mL
	LM103001-J	Elution Buffer	20 mL

4. Preparation before Experiment

4.1. Prepare equipment and reagents: desktop centrifuge, shaker, water bath or metal bath, isopropanol, centrifuge tubes, etc.

4.2. Centrifuge steps: Unless otherwise specified, all centrifuge steps should be performed at room temperature, using a conventional desktop centrifuge that can reach 12,000 rpm.

4.3. Before first use, add RNase A (10 mg/mL) to the buffer RS bottle, mix thoroughly, and label. Store at 4°C after each use.

4.4. Before first use, add 37 mL of anhydrous ethanol to the protein removal solution PL* bottle, mix thoroughly, and label.

4.5. Before first use, add 4 times the volume of anhydrous ethanol to the washing solution W* bottle, mix thoroughly, and label. If the washing solution W* has been significantly mismeasured due to improper transportation or storage, adjust the volume using a graduated cylinder, then add 4 times the volume of anhydrous ethanol. After each use, tightly close the bottle cap to preserve the ethanol content in the washing solution W*.

5. Operation Method

5.1. Sample Pre-treatment

5.1.1. Transfer 150-200 mL of overnight cultured bacterial suspension into a 50 mL centrifuge tube. Centrifuge at 10,000 rpm for 1 minute, discard the supernatant, and collect the bacterial pellet.

Note: For low-copy plasmids or plasmids larger than 10 kb, it is recommended to increase the bacterial volume and proportionally increase the volume of buffer RS*, lysis buffer LB, and binding buffer BD.

5.1.2. Add 7.5 mL of buffer RS*, resuspend the bacterial pellet by pipetting or vortexing.

Note: Ensure the bacterial pellet is thoroughly resuspended.

5.1.3. Add 7.5 mL of lysis buffer LB, gently invert the tube 6-8 times to fully lyse the bacterial cells. Incubate at room temperature for 4 minutes.

Note: The lysis process should be gentle to avoid genomic DNA shearing.

Note: The lysed bacterial solution should appear clear and viscous. If it is turbid, it may indicate incomplete lysis, and you may need to reduce the bacterial amount used.

5.1.4. Add 7.5 mL of binding buffer BD, immediately invert gently 6-8 times to mix thoroughly. Centrifuge at 10,000 rpm for 10-15 minutes, carefully collect the supernatant.

Note: Avoid aspirating any floating white precipitate.

5.1.5. Add 1/10 volume of endotoxin-free solution ER (about 2.4 mL) to the supernatant, invert to mix, and incubate on ice for 5 minutes until the solution becomes clear and transparent (or slightly turbid). During the ice incubation, gently invert 2-3 times to mix.

5.1.6. Let the solution stand at room temperature (25°C) for 5 minutes until it becomes turbid again. Invert gently to mix.

Note: If the temperature is lower than room temperature, a 37°C water bath may be used to restore turbidity.

5.1.7. Centrifuge at 10,000 rpm for 10 minutes at room temperature to separate the layers. The upper aqueous phase contains DNA, and the lower blue oil-like phase contains endotoxins and other substances. Carefully collect the upper aqueous phase into a new centrifuge tube.

5.1.8. Add 0.5 times the volume of isopropanol (about 11 mL, prepare yourself), and mix thoroughly by inverting.

5.2. Plasmid DNA Extraction

5.2.1. Place the DNA adsorption column E1 into a 50 mL collection tube and set aside.

5.2.2. 2)Add the pre-treated mixture into the DNA adsorption column E1, then centrifuge at 10,000 rpm for 1 minute and discard the waste liquid.

Note: The maximum volume of the mixture added at one time is 10 mL, and centrifugation can be done in multiple steps if necessary.

5.2.3. Add 10 mL of protein removal solution PL* and centrifuge at 10,000 rpm for 1 minute, discarding the waste liquid.

Note: Ensure that the protein removal solution PL* contains absolute ethanol.

5.2.4. Place the DNA adsorption column E1 back into the collection tube, add 10 mL of wash solution W*, and centrifuge at 12,000 rpm for 1 minute at room temperature, discarding the waste liquid.

Note: Ensure that the wash solution W* contains absolute ethanol.

5.2.5. Repeat step 4.

5.2.6. Place the DNA adsorption column E1 back into the collection tube and centrifuge at 12,000 rpm for 3 minutes at room temperature to remove any residual wash solution W*. Let the column air dry for 3 minutes at room temperature.

5.2.7. Place the DNA adsorption column E1 into a new 50 mL collection tube. Add 1-2 mL of elution buffer to the center of the DNA adsorption column E1 and let it stand at room temperature for 2 minutes. Then, centrifuge at 10,000 rpm for 2 minutes. Collect the filtrate, which is the DNA solution.

Note: To improve recovery yield, you can: Preheat the elution buffer to 70°C; Reapply the DNA filtrate to the column, allow it to stand at room temperature for 2 minutes, then elute again.

Note: The elution buffer does not contain chelating agent EDTA, so it will not affect downstream applications such as digestion or ligation. Water can also be used for elution, but ensure that the pH is above 7.5, as a low pH may affect elution efficiency.

5.2.8. The plasmid DNA solution can be stored at -20°C for long-term storage.

6. Notes

6.1. Avoid prolonged exposure of reagents to air to prevent volatilization, oxidation, or changes in pH. After use, the lids of all solutions should be tightly closed in a timely manner.

6.2. Pay attention to whether any solution has precipitated or become cloudy (especially in low-temperature environments such as winter). If so, you can re-dissolve by incubating at 37°C until the solution becomes clear, to avoid affecting the effectiveness.

6.3. For your safety and health, please wear a lab coat and disposable gloves during operation.

6.4. This product is for research purposes only!