T-Pro Plasmid Midi Kit (20)

Store at RT

(RB94-YPI020) 20 preps

This product is for laboratory research ONLY and not for diagnostic use.

Description	T-Pro Plasmid Midi Kit use pre-packed anion exchange resin columns are used to purity plasmid DNA from 25-100ml of bacterial cultures. In the process, the modified alkaline lysis method and RNase treatment are used to get cleared cell lysate with minimal genomic DNA and RNA contaminants. Following binding of the plasmid DNA to the column, the contaminants can be washed off with wash buffer. Finally, the purified plasmid DNA is eluted with a high salt buffer and then precipitated with isopropanol for desalting. The entire procedure can be completed in 120 minutes resulting in high purity plasmid DNA without ultracentrifuges, HPLC or other toxic reagent.
Features	Purity equivalent to that obtained by 2X CsCl-gradient centrifugation. Reproducible yields of ultrapure DNA. No toxic reagent.
Applications	* High-purity DNA suitable for use in Plasmid DNA Preparation, * Sequencing, * in Vitro Transcription, * Microinjection, * Restriction Digestion.
Quality Control	The quality of Plasmid Midi Kit is tested on a lot-to-lot basis. The kits are tested by isolation of plasmid DNA from 50ml cultures of E.Coli DH5alpha containing the plasmid pUC19(A600>2 units/ml). More than 120µg of plasmid DNA was quantified with a spectrophotometer. The purified plasmid was used for restriction enzyme digestion with EcoR1 and DNA is checked by agarose gel analysis.
Storage	T-Pro Plasmid Midi Kit is stable for RT

T-Pro Plasmid Midi Kit	
PL1 Buffer	120 ml
PL2 Buffer	120 ml
PL3 Buffer	120 ml
PL4 Buffer	120 ml
PL5 Buffer	320 ml
PL6 Buffer	240 ml
RNase A (50 mg/ml)	240 µl
PM columns	20 pcs

Process: Gravity flow through Ion Exchange resin
Sample
Source: Plasmid DNA from E. coli.
Sample size: Midi: 100-150ml
Capacity: Midi: 500µg
Operation: Gravity-Flow
Operation Time: 120 min

Protocol

Additional requirements:	*	75% Ethanol
	*	Isopropanol
	*	Sterile, DNase-free pipette tips
	*	Sterile, 50ml centrifuge tube
Things to do before starting	*	Use 25~100ml of bacterial culture for Midi kit.
	*	Add provided RNase A to PL1 Buffer and store 4℃
	*	If precipitate have fomed in PL2 Buffer, warm the buffer in a 37℃ waterbath to dissolve them.
Cell Harvesting/Column Equilibration	1	Harvest the bacterial culture 25~100ml by centrifugation at 6,000xg for 15 minutes.
Resuspension	2	Apply 5ml of PL1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
Lysis	3	Add 5ml of PL2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, avoid shearing genomic DNA.
	4	Stand for 5 minutes at room temperature until lysate clears.
Neutralization	5	Add 5ml of PL3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex. Until the precipitate formation, shake a few times to broken it. Incubate at room temperature for 5 minutes to digest RNA.
	6	Centrifuge at 15,000xg for 20 minutes at room temperature.
Column Equilibration	7	Place a PM Column on a 50ml centrifuge tube (not provided).
	8	Equilibrate the PM column by applying 5ml of PL4 Buffer and allow the column to empty by gravity flow.
	9	Discard the filtrate.
DNA Binding	10	Apply the supernatant to the equilibrated PM Column and allow it to flow through by gravity flow.
	11	Discard the filtrate.
Wash	12	Wash the PM Column by applying 15ml of PL5 Buffer and allow the PM Column to empty by gravity flow.
	13	Discard the filtrate.
DNA Elution	14	Place the PM Column in a clean centrifuge tube (not provided), add 12ml of PL6 Buffer to elute DNA by gravity flow.
DNA Precipitation	15	Precipitate DNA by adding 9ml (0.75-folds of PL6 Buffer) of isopropanol to the eluted DNA.
	16	Mix gently and centrifuge at 20,000xg for 30 minutes at 4℃.
	17	Carefully remove the supernatant and wash the DNA Pellet with 9ml of room temperature 75% ethanol.
	18	Centrifuge at 20,000xg for 10 minutes at 4℃.
	19	Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.
	20	Dissolve the DNA pellet in 500µl or a suitable volume of TE or ddH_2O.