T-Pro Plasmid Mini Kit

Store at RT

(RB94-YPD100) 100 preps

(RB94-YPD250) 250 preps

T-Pro Biotechnology

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

Description	The T-Pro Plasmid Mini Kit is designed for rapid isolation of plasmid or cosmid DNA from 1-5ml of bacterial cultures. T-Pro Mini Prep Systems (mini column) have been optimized for reliable yied (T-Pro Midi / Maxi is neccessary for preparative yields of 500µg – 1mg.) The modified alkaline lysis method followed by RNase treatment is utilized to obtain cleared cell lysate with minimal genomic DNA or RNA contamination. Silica spin technology coupled with chaotrophic salt provide a reliable DNA binding and elution system. Purified DNA is ready for restriction digestion, ligestion, PCR and sequencing reactions.
Applications	-Plasmid DNA preparation -Sequencing -In Vitro Transcription -Microinjection
Quality Control	The quality of T-Pro Plasmid Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of plasmid DNA from 4ml cultures of E.Coli DH5alpha transformed with the plasmid pUC19(A600>2 units/ml). More than 20µl of plasmid DNA was quantified with spectrophotometer. 1µl of the purified product is restriction enzyme digestion with EcoR1 and checked by agarose gel analysis.
Storage	T-Pro Plasmid Mini Kit is stable for RT

T-Pro Plasmid Mini Kit		
	RB94-YPD100	RB94-YPD250
PS1 Buffer	25 ml	60 ml
PS2 Buffer	25 ml	60 ml
PS3 Buffer	45 ml	90 ml
W1 Buffer	45 ml	120 ml
W2 Buffer	25 ml	50 ml ***
Elution Buffer	15 ml	30 ml
RNase A (50 mg/ml)	50µl	120µl
2ml Columns	100 pcs	250 pcs
PD columns	100 pcs	250 pcs

Sample Source:
Plasmid DNA from Bacteria.

Sample Size:
1-5ml of LB broth overnight incubate bacterial cultures.

Typical Plasmid Yield:
Low copy 0.5-5 µg
High copy 10-20 µg

Preparation Time:
20 min(s).

*add provided RNase A to PS1 Buffer and store at 4℃

**If precipitate has fomed in PS2 Buffer, warm the buffer in a 37℃ water bath to dissolve.

***Add 100ml (100) / 200ml(250) ethanol (96-100%) to wash buffer prior to initial use.

Protocol (Low Copy Number)

Add ethanol and RNase A to buffers according to component instructions. The typical yield is about 1.0-3.0 µg per 1ml culture when preparing low-copy-number plasmid from overnight bacterial culture in LB medium. If the plasmid is larger than 10 Kb, preheat the Elution Buffer to 70℃ prior to the Elution Step.

Cell Harvesting	1	Harvest up to 10ml of overnight culture by centrifugation.
Resuspension	2	Apply 400µl of PS1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortexing or pipetting.
Lysis	3	Add 400µl of PS2 Buffer and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing genomic DNA.
	4	Allow mixture to stand for 3 min at room temperature until lysate clears.
Neutralization	5	Add 600µl of PS3 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 min to digest RAN.
	6	Centrifuge for 5 min at full speed 13,000xg.
DNA Binding	7	Place a PS Column in a Collection Tube.
	8	Apply 750µl of the clear lysate supernatant from step 6 to the PS Column.
	9	Centrifuge at full speed 13,000 rpm for 30 seconds. Discard the flow-through and place the PS Column back into the Collection Tube.
	10	Apply the remnant clear lysate to the same PS Column.
	11	Centrifuge at full speed 13,000 rpm for 30 seconds. Discard the flow-through and place the PS Column back into the Collection Tube.
Wash	12	Add 400µl of W1 Buffer in the PS Column.
	13	Centrifuge at full speed 13,000 rpm for 30 seconds.
	14	Discard the flow-through and place the PS Column back into the Collection Tube.
	15	Add 600µl of W2 Buffer (ethanol added) in the PS Column.
	16	Centrifuge at full speed 13,000 rpm for 30 seconds.
	17	Discard the flow-through and place the PS Column back into the Collection Tube.
	18	Centrifuge again for 3 min at full speed to dry the column matrix.
Elution	19	Transfer dried PS Column to a clean microcentrifuge tube (not provided).
	20	Add 50µl of (preheated) Elution Buffer or distilled water in the center of the column matrix. (If plasmid DNA is larger than 10kb, use preheated Elution Buffer (70℃) to improve the elution efficiency).
	21	Stand for 2 min until Elution Buffer or distilled water absorbed by the matrix.
	22	Centrifuge for 2 min at full speed 13,000 rpm to elute purified DNA.

Protocol (High Copy Number)

Add ethanol and RNase A to buffers according to component instructions. The typical yield is about 1.0-3.0 μg per 1ml culture when preparing low-copy-number plasmid from overnight bacterial culture in LB medium. If the plasmid is larger than 10 Kb, preheat the Elution Buffer to 70℃ prior to the Elution Step.

Cell Harvesting	1	Transfer 1.5ml of bacterial culture to a microcentrifuge tube (not provided).
	2	Centrifuge for 2 min at full speed (6,000 rpm) in a microcentrifuge and discard the supernatant. (If more than 1.5ml of bacteria culture is used, repeat the Harvesting Step. For over 5ml, use multiple columns.)
Resuspension	3	Apply 200μl of PS1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortexing or pipetting.
Lysis	4	Add 200μl of PS2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, avoid shearing genomic DNA.
	5	Stand for 3 minutes at room temperature until lysate clears.
Neutralization	6	Add 300μl of PS3 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 min to digest RAN.
	7	Centrifuge for 5 min at full speed 13,000xg at room temperature.
DNA Binding	8	Place a PS Column in a Collection Tube.
	9	Apply the clear lysate supernatant from step 7 to the PS Column.
	10	Centrifuge at full speed 13,000 rpm for 30 seconds.
	11	Discard the flow-through and place the PS Column back into the Collection Tube.
Wash	12	Add 400μl of W1 Buffer in the PS Column.
	13	Centrifuge at full speed 13,000 rpm for 30 seconds.
	14	Discard the flow-through and place the PS Column back into the Collection Tube.
	15	Add 600μl of W2 Buffer (ethanol added) to PS Column.
	16	Centrifuge at full speed 13,000 rpm for 30 seconds.
	17	Discard the flow-through and place the PS Column back into the Collection Tube.
	18	Centrifuge again for 3min at full speed 13,000 rpm to dry the column matrix.
Elution	19	Transfer the dried PS Column to a clean 1.5ml microcentrifuge tube (not provided).
	20	Add 50μl of Elution Buffer or distilled water into the center of the column matrix. Avoid residual buffer adhering to the wall of the column (If plasmid DNA is larger than 10kb, use preheated 70℃ Elution Buffer to improve the elution efficiency).
	21	Stand for 2 min until Elution Buffer or distilled water is absorbed by the matrix.
	22	Centrifuge for 2 min at full speed 13,000 rpm to elute purified DNA.
	23	If would like more production, repeat this elution step again.

Problem	Possible Reasons/Solution
Low yield	**Bacterial cells were not lysed completely** Too many bacterial cells were used. If using more than 10 A600 units of bacterial culture, separate into multiple tubes. Following PS3 Buffer addition, break up the precipitate by inverting to ensure higher yield.
	Incorrect W2 Buffer Check to ensure Ethanol was added to W2 Buffer prior to use.
	Incorrect DNA Elution Step Ensure that Elution Buffer was added and absorbed to the center of PS Column matrix.
	Incomplete DNA Elution If plasmid DNA is larger than 10kb, use preheated Elution Buffer (70 ℃) on Elution Step to improve the elution efficiency.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** After wash step, dry PS Column with additional centrifugation at top speed for 5 min or incubation at 60℃ for 5min.
	RNA contamination Prior to using PS1 Buffer, ensure that RNase A was added. If RNase A added PS1 Buffer is out of date, add additional RNase A. Too many bacterial cells were used, reduce sample volume.
	Genomic DNA contamination Do not use overgrown bacterial culture. During PS2 and PS3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination If host cells have high nuclease activity (e.g. and A+ strains), perform this Optional Wash Step to remove residual nuclease. After DNA Binding Step, and 200µl of PS3 Buffer into PS column and incubate for 2 min at room temperature. Centrifuge at 13,000rpm for 30 seconds. Continue from standard Wash Step.