Plasmid Purification Kit

Catalog No.: K003

Size: 50 T/200 T

Storage: Store at room temperature for one year except for Buffer P1 at 2-8℃ and RNase A at -20℃.

Kit Contents :

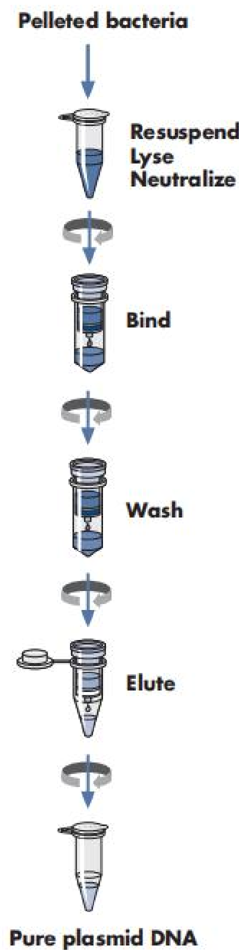
Size	50T	200T	Storage
Buffer P1	15 ml	60 ml	2-8°C
Buffer P2	15 ml	60 ml	RT
Buffer N3	20 ml	80 ml	RT
Buffer PB	25 ml	100 ml	RT
Buffer PE	15 ml	60 ml	RT
Buffer EB	10 ml	30 ml	RT
RNase A(10 mg/ml)	150 ul	600 ul	-20°C
Spin Column CM	50 pcs	200 pcs	RT

Safety Information :

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Introduction:

FineTest Plasmid Purification Kit is designed to extract high-quality plasmid DNA for molecular biology experiments in eukaryotic transfection and in vitro expression. The kit bases on SDS- alkaline lysis method and provides a rapid operation for extraction and purification by using silica- membrane binding columns. Using the kit, plasmid DNA can be isolated in less than 1 hour with purity of A260/A280>1.86, greatly reducing the time spent on extraction and achieving high purification.



Preparation :

1. Add the isolated packed RNase A to buffer P1 and mix, the mixture should be stored at 2-8°C and used up in 3 months.
2. Add ethyl alcohol to PE Buffer bottle to make the ethanol final concentration 80% v/v.
3. For each assay sample, one 1.5ml centrifuge tube, 1 column and 1 collection tube should be prepared.

Procedure :

1. Add 1.5 ml of the culture to a centrifuge tube and centrifuge for 1 min at 12,000 rpm and discard the supernatant.
2. Add 250 µl buffer P1 (including RNase A) to the centrifuge tube and resuspend the bacteria cells. Note: Ensure that no cell clumps should be visible after resuspension of the pellet.
3. Add 250µl buffer P2 to the centrifuge tube. Mix by inverting the tube gently for 6 to 8 times. Note: Step 3 must be dealt gently or DNA string may be cracked. The centrifuge tube can be inverted more than 6 times until the solution is clear if necessary, and make sure that the reaction period should be limited in 5 min.
4. Add 350µl buffer N3. Cap and mix by inverting the tube gently for 6 to 8 times.
5. Centrifuge the tube for 10 min at 12,000 rpm and a white spherical particle should appear.
6. Transfer the supernatant to a column-collection tube and centrifuge the column-collection tube at 12,000 rpm for 1 min, then discard the filtered solution.
7. Wash the the column-collection tube with PB Buffer. Add 500µl PB Buffer to the column- collection tube and centrifuge at 12,000 rpm for 30~60 sec, then discard the filtered solution.

8. Wash the the column-collection tube with PE Buffer. Add 750μl PE Buffer to the column- collection tube and centrifuge at 12,000 rpm for 30~60 sec, then discard the filtered solution.
9. Repeat the step 8 to wash the column-collection tube again.
10. Eliminate the residual PE Buffer. Centrifuge the step 9 column-collection tube at 12,000 rpm for 2 min and let the column uncapped and exposed in the air for 5 min to volatilize the PB Buffer.
11. Note: The residual PE Buffer may inhibit the following reaction and lead to extraction failure.
12. Transfer the spin column into a clear centrifuge tube. To elute DNA, add 50-100 μl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the column membrane ， let the column stand for 1 min, and then centrifuge the column for 1 min.

IMPORTANT :

Ensure that the elution buffer is dispensed directly onto the column membrane for complete elution of bound DNA. Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Determination of yield :

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. For reliable spectrophotometric DNA quantification, A260 readings should lie between 0.1 and 1.0.

Agarose gel analysis :

We recommend removing and saving aliquots during the purification procedure. If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine the stage of the purification procedure where the problem occurred.

Extraction Example :

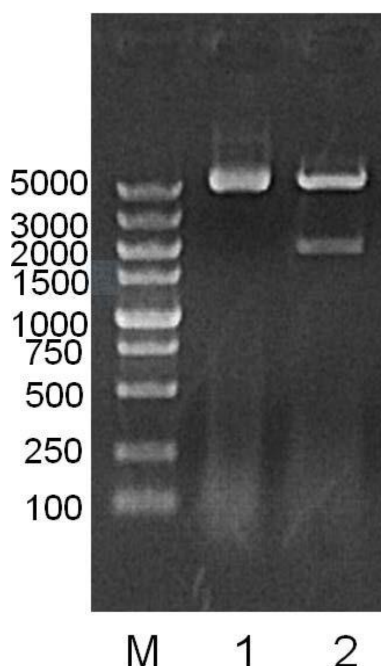


Fig. 1 Example: pET28a-X extracted by using Fine Plasmid Purification Kit.

Lane M: DNA Marker Lane 1: pET28a-X

Lane 2: pET28a-X digested by two restriction enzymes

Troubleshooting :

Problem	Cause	suggestions
Low DNA yields	Poor Cell Lysis	Cells may not have been dispersed adequately prior to the addition of buffer P2. Make sure to vortex cell suspension to completely disperse.
		Increase incubation time with buffer P2 to obtain a clearly lysate.
	Bacterial Clone is over grown or not fresh.	Do not incubate cultures for more than 16 hours at 37°C.Storage of cultures for extended periods prior to plasmid isolation is detrimental.
	Low elution efficiency	The pH of Elute Buffer or water must be≥8.0 .
No DNA Eluted	TE Buffer is not diluted with absolute ethanol.	Prepare DNA Wash Buffer Concentrate according to Preparation 2.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of buffer P2.	Do not vortex or mix aggressively after adding buffer P2.
RNA visible on agarose gel	RNase A not added to buffer P1.	Check that RNase A provided with the kit has been used. If buffer P1 is 6 months old, add more RNase A.
Plasmid DNA floats out of well while loading agarose gel	Ethanol has not completely been removed from spin column in step 10.	Expose spin column in the air for more minutes to dry the column before elution.