Enzymax, LLC

Product Information

Product Name: DNA Tini prep Kit
Catalog no.: 600
Size: 50 prep

Related products:		Catalog No.
DNA mini-spin columns (30-50µl vol.)		EZC101
DNA Tini-spin columns (5-20µl vol.)		EZC106
DNA gel extraction kit with Tini column	(50 preps)	604

Description:

This kit utilizes the Tini spin column to purify plasmid DNA directly from single colony or cultured cells from 10^1-10^5. The unique design of the column has eliminated problems associated with dead volume and buffer retention, resulting in increased DNA/RNA recovery and purity, as well as minimizing the elution volume (as low as 5µl). It is the easiest method for isolation of plasmid DNA and produces high-yield plasmid DNA of automated sequence quality. The recovered plasmid DNA has a 1.8-2.0 $OD_{260/280}$ ratio and is ready for automated sequencing, PCR, restriction digestion, or other downstream applications. The purified plasmid DNA is primarily in the supercoiled form.

Kit Contents:

Components	*DNA Tini prep 50 Preps (cat#600)*
Solution I:	6ml
Solution II:	12ml
Solution III:	25ml
5xWash Buffer*:	20ml
Elution Buffer	5ml
Tini spin column with collection tube	50

***Add 100% ethanol before use:** add 80ml 100% ethanol to 20ml 5xWash buffer.
***Solution II may form a precipitate upon storage. Warm the solution at 37°C.**
***Tini Spin columns can be order separately for leftover solution (cat#EZC106, $39 for 50 columns)

Storage:

Store all Buffers at room temperature; Solution I may be refrigerated for long-term storage.

Caution:

Solution III contains chaotropic salt. Please use proper safety precautions and always wear gloves when handling the reagent. Avoid contact with skin, eyes or clothing. In case of accidental spill or contact, wash thoroughly with water, seek medical advice if necessary. For **Solution II,** avoid contact with skin and eyes. Do not inhale or swallow.

Protocol

This protocol is designed for plasmid DNA isolation directly from single colony on E.coli plate. For small scale plasmid DNA isolation from cultured cells (10^1-10^5), you can adapt the protocol depending on the cell numbers by increasing the amount of Solution I, II, and, III proportionally. For example, if you add 50µl of Solution I to the cell pellet, you need to add 100µl of Solution II and 175µl of Solution III. You will need to add 200µl of Wash buffer in the washing step.

Procedures: Add 80ml of 100% ethanol to 20ml **Wash Buffer** before the procedures.

1. Pick single colony from plate (~2mm size) with pipette tip and resuspend in 15µl of **Solution I.**
2. Add 30µl of **Solution II** and immediately mix gently by tapping the tube with your finger.
3. Neutralize by adding 52.5µl of **Solution III** and mix gently by tapping the tube with your finger.
4. Centrifuge for 5 minutes at full speed (>10,000 rpm) in a microcentrifuge.
5. Transfer supernatant to the **Tini Spin Column**, centrifuge 1 minute, and discard the flow-through.
6. Add 300µl **Wash Buffer** to the column, centrifuge at full speed for one minute, and discard the flow-through. Repeat this step one more time.
7. Centrifuge the tube with the lid open for 2 minute to remove residual ethanol in the **Wash Buffer**. (**Note:** this step is important since ethanol may affect downstream applications)
8. Transfer the column to a clean microcentrifuge tube (not supplied in the kit).
9. Add 5-15µl of **Elution Buffer** or ddH_2O (not supplied in the kit) to the center of each spin column**,** and centrifuge at full speed for one minute to elute the genomic DNA from the column.
   Note: For better DNA yield, pre-warm the elution buffer (or ddH_2O) at 65*C for 5 min. and then add it to the center of the column and leave at room temperature for 5 min before centrifuging.