Enzymax, LLC

Product Information

Product Name: EZ DNA Miniprep Kit
Catalog no.: EZC204
Size: 50 preps

Related products:	Catalog No.
DNA mini spin columns (30-50µl vol.)	EZC101
DNA Tini spin columns (5-20µl vol.)	EZC106
DNA gel extraction kit	EZC203

Description:

This kit utilizes the silica spin filter to purify plasmid DNA. 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, restriction digestion, or other downstream applications. The purified plasmid DNA is primarily in the supercoiled form. If you only need small amount of DNA samples for your downstream applications, you can also use Tini spin column (cat# EZC106, DNA yield from10ng-20µg) to replace the mini spin column but only use 1/3-1/5 of the solutions.

Kit Contents:

Components	*EZ DNA Clean-up 50 Preps* (cat# EZC204)
Solution I	6ml
Solution II**	12ml
Solution III	25ml
RNase A	1 vial (120µl)
5xWash Buffer*	20ml
Elution Buffer	5ml
Mini 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.**
***Mini Spin columns can be order separately for leftover solution (cat#EZC101, $39 for 100 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.

Protocol

1. **Prepare solutions:**
• Add 1 vial of **RNase A** into **solution I,** and keep at *4*°C or *20*°C for long term storage.
• Add 80ml of 100% ethanol to 20ml **Wash Buffer**.
2. **Pellet bacteria:**
• Centrifuge 1-3ml of bacterial culture grown overnight in the presence of the appropriate antibiotic. Remove all growth medium from the pellet.
3. **Lysis and neutralization:**
• Add 100µl of **Solution I** to each sample tube and vortex vigorously. Pellets must be completely dissociated for maximum yield.
• Add 200µl of **Solution II** and immediately invert 4-6 times gently to mix and keep at room temperature for 1min. Do not vortex.
• Neutralize by adding 350µl of **Solution III**. Gently invert the tube for 4-6 times and keep at room temperature for 1min. Do not vortex. Centrifuge the tube for 5 minutes at full speed (>10,000 rpm) in a microcentrifuge.
4. **DNA Binding:**
• Transfer supernatant to the **Spin Column**, centrifuge 2 minutes, and discard the flow-through.
5**. Wash:**
• Add 750µl **Wash Buffer** to the column, centrifuge at full speed for 2 minutes, and discard the flow-through.
• Repeat the above wash step once. Discard the flow-through.
• Cut off the cap on the column and centrifuge for 2 minute to remove residual ethanol. (**Note:** this step is important since ethanol may affect downstream applications)
• Transfer the column to a clean microcentrifuge tube (not supplied in the kit).
6. **Elution:**
• Add 30-50µl 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.