dNEAT™ Plasmid Purification Kit

PURK-MIN-050

Description

The dNEAT™ Plasmid Purification Kit offers a rapid and convenient method for the isolation of high-quality plasmid preparations in mini format. The kit is based in DNA ability to bind silica in the presence of high concentrations of chaotropic salts. dNEAT™ spin columns have an exclusive silica membrane and it binds up to 20 µg DNA.

Features

- ✓ High yields of up to 24 µg (in 5% of isolations) of DNA suitable for all molecular biology procedures
- ✓ No phenol-chloroform extraction
- ✓ Ready to use plasmid DNA
- ✓ Just a few minutes procedure

Applications

All molecular biology applications, such as:

- ✓ Digestion with restriction enzymes
- ✓ Automated sequencing
- ✓ PCR template
- ✓ Bacterial transformation
- ✓ Transfection

Storage

The dNEAT™ Plasmid Purification Kit should be stored at room temperature (15–25°C) for up to 12 months without any reduction in performance.

Store the RNase A at -20ºC. After addition of RNase A to Buffer S-I reagent can be stored at 4ºC.

Quality Certification

The dNEAT™ Plasmid Purification Kit is tested for the isolation of any plasmid DNA from transformed *E. coli*. The quality of purified DNA is analysed by:

- ✓ Ratio 260/280
- ✓ Agarose gel electrophoresis
- ✓ Digestion with restriction endonucleases

Kit Components

dNEAT™ minispin columns	50 pcs
Collection tubes (2 mL)	50 pcs
S-I Buffer	16 ml
S-II Buffer	16 ml
S-III Buffer	16 ml
Binding Buffer	30 ml
Washing Buffer*	8 ml
Elution buffer	10 ml
RNase A	160 µl

*Ethanol (96%-100%) [not included] must be added prior to use as indicated on the label. After ethanol has been added, mark the bottle to indicate that this step has been completed

Product use limitation

This product is developed, designed and sold exclusively for research purposes and use only. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals.

Protocol

Preparation of working solutions

Before starting the protocol prepare the following reagent:

- ✓ To prepare the S-I Buffer add 160 µl of RNAse A
- ✓ Add the volume ethanol (96%-100%) specified [Not included] to Washing Buffer prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed

Detailed Protocol

1. Pour the culture in a 1.5 ml centrifuge tube and harvest the bacterial cells by centrifugation at 13000 rpm for 2 minutes. For low-copy plasmids 3 ml of culture should be collected 2 volumes of each solution to obtain good yields
2. Resuspend the bacterial pellet in 100 µL of Buffer S-I
3. Add 100 µL of Buffer S-II, mix thoroughly by inverting the tube 6 times
4. Add 100 µL of Buffer S-III, mix thoroughly by inverting the tube 8 times
5. Centrifuge at 13000 rpm for 10 min. Recover the supernatant containing plasmid DNA promptly into a 1.5 ml centrifuge tube
6. Add 500 µL of Binding Buffer, mix by inverting the tube several times. Incubate at room temperature for 5 min
7. Transfer the supernatants from step 6 to the dNEAT™ spin column by decanting or pipetting
8. Centrifuge at 5500 rpm (9500g) for 90 seconds. Discard the flow-through
9. Wash the dNEAT™ spin column by adding 700 µL of Washing Buffer and centrifuging at 5500 rpm for 90 s. Discard the flow-through
10. Place the dNEAT™ spin column in a collection tube and add 500 µL of Isopropanol pure
11. Centrifuge at 5500 rpm for 90 s. Discard the flow-through
12. Centrifuge at 13000 rpm for 90 s. This step helps remove traces of isopropanol
13. Place the dNEAT™ spin column into a new, labelled 1.5 microcentrifuge tube and pipet 50-60µl of Elution Buffer directly into the membrane. Close the cap and incubate for 1 minute at room temperature
14. Centrifuge at 13000 rpm for 1 minute to elute DNA