Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Plasmid DNA Extraction Kit

(Catalog# K1445-100; 100 tests; Storage at Multiple Temperatures)

I. Introduction:

Plasmid DNA extraction and purification kit with proprietary separation and buffer systems, allows extracting and purifying plasmid DNA from 2.0~5.0 mL of bacteria culture. The typical yield is 10~20 µg of high quality plasmid DNA and the typical OD260/280 between 1.75 to 1.85. Purified plasmid DNA can be directly used for a variety of molecular biology applications such as enzymatic digestion, sequencing and transformation.

The kit will work with a 48 well round bottom plates if a special magnetic frame is used. The kit can also be used with a variety of automatic nucleic acid extraction instruments or workstations.

II. Applications:

- Extraction and purification of plasmid DNA from bacteria culture. Purified DNA can be used for enzymatic digestion, sequencing, transformation etc.

III. Sample Type:

- Bacterial cultures

IV. Kit Contents:

Component	K1445-100	Part Number
Magnetic Beads	5 mL	K1445-100-1
RNase A Solution	55 µl	K1445-100-2
Suspension Solution	22 ml	K1445-100-3
Lysis Solution	22 ml	K1445-100-4
Neutralization Solution	40 ml	K1445-100-5
Wash Solution*	38 ml	K1445-100-6
Elution Buffer	10 ml	K1445-100-7

**** Add 25 ml of Isopropanol to Wash Solution* (K1445-100-6) before use.***

V. User Supplied Reagents and Equipment:

- 80% Ethanol in water.
- Magnetic racks compatible with vials.
- Isopropanol (ACS grade).

VI. Storage Conditions and Reagent Preparation:

Magnetic beads should be stored at 2-8°C but other kit reagents need to be stored at room temperature. Lysis solution may turn cloudy if stored in the cold room. To clear it up place the bottle in a water bath at 37°C.

Reagent Preparation:

Wash solution* (K1445-100-6): Dilute the solution by adding 25 ml of Isopropanol before using.

VII. Assay Protocol:

1. Preparation. Before the first use, add all the RNase A solution into the Suspension Solution.
2. Harvest cells. Spin 1.5-5 mL of overnight grown bacterial cells at 12,000 rpm for 2 min.
3. Re-suspend the cells. Remove culture media completely. Add 200 µl of suspension solution (with RNase A), then vortex to ensure complete suspension of cell. Transfer cells to a clean Eppendorf tube.
4. Lyse the cells. Add 200 µl of Lysis solution and mix by inverting the tube for 6-8 times. Incubate for 2 min. Do NOT vortex.
5. Neutralize the solution. Add 350 µl of Neutralization solution and mix by inverting the tube for 8-10 times. Do NOT vortex.
6. Spin the tube at 12,000 rpm for 15 min at 4°C.
7. Transfer the solution to a clean Eppendorf tube, then add 50 µl of magnetic beads, mix well and incubate 3-5 min at RT. Put Eppendorf tube onto the magnet rack for 20 seconds.
8. Remove solution by holding the magnet rack upside down or by pipetting.
9. Wash the beads with 500 µl of Wash Solution and then repeat Step 8.
10. Wash the beads with 500 µl of 80% ethanol twice repeating Step 8.
11. Dry the beads at 55°C for 8 min leaving the tube open. Do not over-dry the beads.
12. Elute the DNA from beads with 50-100 µl of elution buffer, incubate for at least 2 min and then vortex at full speed for 1 min. alternatively, incubation at 60°C for 2 min may improve the recovery for DNA larger than 10 kb.
13. Remove beads by using magnet rack, pipette DNA out and transfer to a clean tube.
14. Store purified DNA at -20°C for long-term storage.

VIII. Related Products:

- Plasmid Miniprep Kit (# K529)
- Plasmid Miniprep Kit I (# K1312)
- Plasmid Midi Kit II (# K1315)
- Plasmid Midi Kit I (# K1314)
- Plasmid Miniprep Kit II (# K1313)

FOR RESEARCH USE ONLY! Not to be used on humans.