

peqGOLD Bacterial DNA Kit

Product	13-3450-00	13-3450-01	13-3450-02
Purifications	5	50	200
peqGOLD DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
TL Buffer	1.5 mL	20 mL	50 mL
BL Buffer	2 mL	20 mL	50 mL
HBC Buffer	5 mL	25 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	15 mL	60 mL
Glass Beads S	150 mg	2 g	8 g
Proteinase K Solution	150 µL	1.5 mL	6 mL
RNase A	30 µL	275 µL	1.1 mL
Lysozyme	5 mg	50 mg	4 x 50 mg

Supplied by user:

- Microcentrifuge capable of 13,000 x g
- Centrifuge to pellet culture capable of 4,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath capable of 37°C
- Shaking water bath capable of 55°C
- Incubator or water bath capable of 65°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- TE Buffer

Before starting:

- Prepare Lysozyme, HBC Buffer, and DNA Wash Buffer according to the directions on the bottles.
- Set a water bath to 37°C.
- Set a shaking water bath to 55°C.
- Set water bath, heat block, or incubator to 65°C.
- Heat Elution Buffer to 65°C.

DNA Extraction and Purification from up to 3 mL LB Culture

1. Culture bacteria in LB media to log-phase. (Overnight culture can be used in many cases.)
2. Centrifuge no more than 3 mL culture or 1 x 10⁹ cells at 4,000 x g for 10 minutes at room temperature. Aspirate and discard the media.
3. Add 100 µL TE Buffer. Vortex to completely resuspend the pellet.
4. Add 10 µL Lysozyme resuspended with Elution Buffer (see bottle for instructions). Incubate at 37°C for 10 minutes. The amount of enzyme required and/or the length of incubation may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield better results.

Optional: Follow the short protocol below for difficult to lyse bacteria.

1. Add 25 mg Glass Beads S to a 1.5 mL microcentrifuge tube (not provided).
2. Add sample to the Glass Beads S.
3. Vortex at maximum speed for 5 minutes.
4. Let sample stand to allow the beads to settle.
5. Transfer supernatant to a new 1.5 mL microcentrifuge tube.
5. Add 100 µL TL Buffer and 20 µL Proteinase K Solution. Vortex to mix thoroughly. Incubate at 55°C in a shaking water bath. Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate the samples and shake or briefly vortex every 20-30 minutes.
6. Add 5 µL RNase A. Invert tube several times to mix. Let sit at room temperature for 5 minutes.
7. Centrifuge at 10,000 x g for 2 minutes to pellet any undigested material. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. Do not disturb the pellet.

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8. Add 220 μ L BL Buffer. Vortex to mix thoroughly. Incubate at 65°C for 10 minutes. A wispy precipitate may form upon addition of BL Buffer; it does not interfere with DNA recovery.
9. Add 220 μ L 100% ethanol. Vortex for 20 seconds at maximum speed to mix thoroughly. Break any precipitates by pipetting up and down 10 times.
10. Insert a peqGOLD DNA Mini Column into a 2 mL Collection Tube. Transfer the entire sample to the peqGOLD DNA Mini Column, including any precipitate that may have formed.
11. Centrifuge at 10,000 x *g* for 1 minute. Discard the filtrate and the collection tube.
12. Transfer the peqGOLD DNA Mini Column into a new 2 mL Collection Tube.
13. Add 500 μ L HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at 10,000 x *g* for 1 minute. Discard the filtrate and reuse the collection tube.
14. Add 700 μ L DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at 10,000 x *g* for 1 minute. Discard the filtrate and reuse the collection tube.
15. Repeat Step 14 for a second DNA Wash Buffer wash step.
16. Centrifuge the empty peqGOLD DNA Mini Column at maximum speed ($\geq 10,000$ x *g*) for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.
17. Insert the peqGOLD DNA Mini Column into a new nuclease-free 1.5 mL microcentrifuge tube.
18. Add 50-100 μ L Elution Buffer heated to 65°C. Let sit for 3-5 minutes at room temperature. Yields may be increased by incubating the column at 65°C. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA.
19. Repeat Step 18 for a second elution step.
20. Store eluted DNA at -20°C.