Quick-Start Protocol April 2016

# Ultrapure 100 Buffer Set

The Ultrapure 100 Buffer Set (cat. no. 11910) can be stored at room temperature (15–25°C) for up to 2 years if not otherwise stated on label.

## Further information

- *Ultrapure 100 Handbook*: **www.qiagen.com/HB-1208**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- Add RNase A to Buffer P1 to a final concentration of 100 µg/ml.
- Prechill Buffer P3 to 4°C.
- Equilibrate equipment and buffers except Buffer P3 to room temperature (15–25°C).
- For endotoxin-free DNA, prepare endotoxin-free 70% ethanol just before use by adding 96–100% ethanol to endotoxin-free water.
- Buffers should be prepared as described in the handbook or purchased as the Ultrapure 100 Buffer Set.

**Table 1. Buffer volumes required for preparing DNA from an overnight LB culture using the Ultrapure 100 column procedure***

| | | Buffer | | | |
|---|---|---|---|---|---|
| **Culture volume** | **Pellet wet weight** | **P1, P2 and P3** | **QBT** | **QC** | **QF and QN** |
| 10 l | 33 g | 0.5 l | 350 ml | 3.0 l | 400 ml |
| 20 l | 60 g | 1.0 l | 350 ml | 3.0 l | 400 ml |

* Expected plasmid DNA yields are 3–5 µg/ml LB for high-copy-number plasmids (e.g., pBluescript®, pUC, pTZ, pGEM®) and 0.1–2 µg/ml LB for low-copy-plasmids (e.g., pBR322, cosmids).

1. Resuspend the bacterial pellet in 1000 ml Buffer P1 containing RNase A.

2. Add 1000 ml Buffer P2, mix by gently inverting the bottle 5–6 times and incubate at room temperature (15–25°C) for up to 5 min.
3. Add 1000 ml of chilled Buffer P3, mix by inverting the bottle 5–6 times and incubate on ice for at least 30 min.
4. Centrifuge at 4°C for at least 30 min at 11,300–17,700 x *g*. Transfer the supernatant promptly to a fresh vessel.
5. Filter the supernatant through a premoistened, folded filter. During this step, incubate the cleared lysate on ice.
6. **Optional**: To prepare endotoxin-free plasmid DNA, add 1/10 volume of Buffer ER to the cleared lysate and incubate for 1 h on ice.
7. Equilibrate the column with 350 ml Buffer QBT at a flow rate of 10–20 ml/min. During this step, incubate the cleared lysate on ice.
8. Connect the inlet tube of the column to the silicon tube of the peristaltic pump. Make sure that all fittings are secure.
9. Load the cleared lysate from step 5 or 6 onto the column at a flow rate of 4–25 ml/min.
10. Wash the column with 3.0 l Buffer QC at a flow rate of 20–30 ml/min.
11. Elute the plasmid DNA with 400 ml Buffer QN at a flow rate of 10 ml/min.
12. Precipitate the DNA with 0.7 volumes of room-temperature isopropanol and centrifuge at 4°C at 20,000 x *g* for 30 min or at 10,000 x *g* for 60 min.
13. Carefully remove the supernatant, wash the DNA with 20 ml room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 20 min at 4°C.
14. Air-dry for approximately 20 min, and redissolve in a suitable volume of buffer (e.g., endotoxin-free Buffer TE).

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