Quick-Start Protocol April 2016

R.E.A.L.® Prep 96 Plasmid Kit

The R.E.A.L. Prep 96 Plasmid Kit (cat. nos. 26171 and 26173) can be stored at room temperature (15–25°C) for up to 12 months if not otherwise stated on label.

Further information

- *R.E.A.L. Prep 96 Handbook*: **www.qiagen.com/HB-2107**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Add RNase A solution to Buffer R1, mix and store at 2–8°C.
- **Required**: QIAvac 96 and a centrifugation system suitable for 96-well blocks.
- Place a QIAfilter 96 plate (yellow) in the top plate of the QIAvac 96 manifold. Place a new square-well block into the base and reassemble the manifold.
- Symbols: ● plasmid or cosmid (96-well); ▲ BAC or cosmid (48-well).

Table 1. Protocol recommendations

	High-copy plasmids	Low-copy plasmids and cosmids	Cosmid (alternative)	BAC, PAC, P1
Block for cultivation	96-well	96-well	48-well	48-well
Media	LB	2x YT	2x YT	2x YT
Volume media per well, ml	1.3	1.3	2.5	2x 2.5* or 2.5
Cultivation time, h	20–24	20–24	16	16–24
Shaker setting, rpm	220	220	175	175
Volume for DNA resuspension, µl	50–250	50	50	22

* Recommended for sequencing; alternative procedure required (see Appendix F of the handbook).

1. Cover the block with adhesive tape and pellet bacterial cells by centrifugation for ● 5 min at 1500 x *g* or ▲ 10 min at 2500 x *g* in a centrifuge with a rotor for 96-well plates. Remove medium by inverting the block.
2. Resuspend each bacterial pellet in 0.3 ml Buffer R1. Dry the top of the block, seal the block with new tape and mix by vortexing.
3. Add 0.3 ml Buffer R2 to each well, seal the block with new tape, mix gently but thoroughly by inverting 10 times or until the lysate is clear and incubate at room temperature (15–25°C) for 5 min.
4. Add 0.3 ml Buffer R3 to each well, seal the block with new tape and mix by inverting ● 10 times or ▲ 20 times, and ▲ incubate on ice for 20 min until the lysate is clear.
5. Transfer the lysates to the wells of the QIAfilter 96 plate on the QIAvac 96.
6. Apply –200 to –300 mbar vacuum until all lysates have passed through.
7. Remove the square-well block containing the cleared lysates from the vacuum manifold. Add 0.7 volumes of room-temperature isopropanol to each well (0.63 ml for 0.9 ml lysate), seal the block with new tape and mix by inverting 3 times.
8. Centrifuge the block at ● 2500 x *g* for 15 min or ▲ 6000 x *g* for 30 min at room temperature. Remove the supernatants by quickly inverting the block over a waste container, then tapping the block firmly, upside down, onto a paper towel.
9. Wash each DNA pellet with 0.5 ml 70% ethanol. Centrifuge the block (in the same orientation as before) at ● 2500 x *g* for 2 min or ▲ 6000 x *g* for 15 min. Remove the wash solutions by inverting the block, then tapping it firmly, upside down, onto a paper towel. ● Air dry the pellets for 15 min or dry under vacuum for 10 min or ▲ air dry pellets for 15 min or until no ethanol remains.
10. Redissolve the DNA in 10 mM Tris·Cl, pH 8.5 as indicated in Table 1 ▲ by incubating overnight at room temperature. Avoid repeated pipetting.

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