QIAFilter Plasmid Midi and Maxi Kits Protocol

Before staring make sure that RNAse A has been added to P1 buffer. Check P2 Buffer for SDS precipitation. Pre-chill P3 buffer at 4°C.

▲ stands for MidiPrep kit; ● stands for MaxiPrep kits

Starting volumes		
High copy vector	**50 mL**	**150 mL**
Low copy vector	**150 mL**	**500 mL**

1) Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).
   Use a tube or flask with a volume of at least 4 times the volume of the culture.
2) Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate **▲ 50 ml** or **● 150 ml** medium with **▲ 25–50 µl** or **● 100–200 µl** of starter culture. For low-copy plasmids, inoculate **▲ 150 ml** or **● 500 ml** medium with **▲ 100–200 µl** or **● 250–500 µl** of starter culture. Grow at 37°C for 12–16 h with vigorous shaking.
3) Harvest the bacterial cells by centrifugation at 3500 rpm for 10 min at 4°C.
   If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.
4) Resuspend the bacterial pellet in **▲ 6 ml** or **● 10 ml** Buffer P1.
   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
5) Add **▲ 6 ml** or **● 10 ml** Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min.
   Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2.

During the incubation, prepare the QIAFilter Cartridge: screw the cap onto the outlet nozzle of the QIAFilter.

6) Add ▲ **6 ml** or ● **10 ml** of chilled Buffer P3, mix immediately and thoroughly by vigorously inverting 4–6 times.

If the mixture still appears viscous, more mixing is required to completely neutralize the solution. If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless.

7) Pour the lysate into the barrel of the QIAFilter Cartridge. Incubate at room temperature for 10 minutes. Do not insert the plunger.

8) Equilibrate a ▲ **QIAGEN-tip 100** or ● **QIAGEN-tip 500** by applying ▲ **4 ml** or ● **10 ml** Buffer QBT, and allow the column to empty by gravity flow.

Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

9) Remove the cap from the QIAFilter Cartridge outlet nozzle. Gently insert the plunger into the ▲ **QIAGEN-tip 100** or ● **QIAGEN-tip 500** Cartridge and filter the lysate into the previously equilibrated Cartridge.

10) Allow the clear lysate to enter the resin by gravity flow.

11) Wash the QIAGEN-tip with ▲ **2 x 10 ml** or ● **2 x 30 ml** Buffer QC.

Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid DNA preparations.

12) Elute DNA with ▲ **5 ml** or ● **15 ml** Buffer QF.

Collect the eluate in a 15 ml or 50 ml tube (not supplied).

13) Precipitate DNA by adding ▲ **3.5 ml** or ● **10.5 ml** (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x g for 30 min at 4°C. Carefully decant the supernatant.

Alternatively, the eluate can be dispatch in 1.5/2 mL microcentrifuge tubes before addition of 0.7 volume of isopropanol. Pellet is then wash with 1 mL 70% ethanol before being dried.

14) Wash DNA pellet with ▲ **2 ml** or ● **5 ml** of room-temperature 70% ethanol, and centrifuge at ≥15,000 x g for 10 min. Carefully decant the supernatant without disturbing the pellet.

15) Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer.