HiSpeed 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 ▲ **HiSpeed Midi** or ● **HiSpeed Maxi** Tip 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 ▲ **HiSpeed Midi** or ● **HiSpeed Maxi** Tip 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 HiSpeed 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 and incubate at room temperature for 5 minutes.

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) During the incubation, remove the plunger from a ▲ 20 ml or ● 30 ml syringe and attach the ▲ QIAprecipitator Midi Module or the ● QIAprecipitator Maxi Module onto the outlet nozzle. Do not use excessive force, bending or twisting to attach the QIAprecipitator.

15) Place the QIAprecipitator over a waste bottle, transfer the eluate/isopropanol mixture into the ▲ 20 ml or ● 30 ml syringe, and insert the plunger. Filter the eluate/isopropanol mixture through the QIAprecipitator using constant pressure.

Important: *Complete the QIAprecipitator procedure (steps 16–21) within 10 min. To prevent detachment of the QIAprecipitator and subsequent loss of DNA and alcohol, do not use excessive force when pushing liquid through the QIAprecipitator.*

16) Remove the QIAprecipitator from the ▲ **20 ml** or ● **30 ml** syringe and pull out the plunger. Re-attach the QIAprecipitator and add 2 ml 70% ethanol to the syringe. Wash the DNA by inserting the plunger and pressing the ethanol through the QIAprecipitator using constant pressure.

17) Remove the QIAprecipitator from the ▲ **20 ml** or ● **30 ml** syringe and pull out the plunger. Attach the QIAprecipitator to the ▲ **20 ml** or ● **30 ml** syringe again, insert the plunger, and dry the membrane by pressing air through the QIAprecipitator quickly and forcefully. Repeat this step.

18) Dry the outlet nozzle of the QIAprecipitator with absorbent paper to prevent ethanol carryover.

19) Remove the plunger from a new 5 ml syringe and attach the QIAprecipitator onto the outlet nozzle. Hold the outlet of the QIAprecipitator over a 1.5 ml collection tube. Add 1 ml of 10 mM Tris pH = 7.4 to the 5 ml syringe. Insert the plunger and elute the DNA into the collection tube using constant pressure.

Ensure that the outlet of the QIAprecipitator is held over the collection tube when Buffer TE is poured into the syringe, as eluate can drip through the QIAprecipitator before the syringe barrel is inserted.

Be careful, as residual elution buffer in the QIAprecipitator tends to foam when expelled.

Alternatively, if a higher DNA concentration is desired and a reduction in yield of up to 10% is acceptable, elute with 500 µl Buffer TE. Lower volumes of elution buffer are not recommended, since incomplete wetting of the QIAprecipitator membrane will lead to reduced DNA yields.

TE Buffer provided in the kit contains 1 mM EDTA that may inhibit further enzymatic reactions*.*

20) Remove the QIAprecipitator from the 5 ml syringe, pull out the plunger and reattach the QIAprecipitator to the 5 ml syringe.

21) Transfer the eluate from step 19 to the 5 ml syringe and elute for a second time into the same 1.5 ml tube.

This re-elution step ensures that the maximum amount of DNA in the QIAprecipitator is solubilized and recovered.