Quick-Start Protocol December 2023

HiSpeed® Plasmid Mega/Giga EF Kits

QIAfilter Cartridges and QIAGEN-tips can be stored for 2 years at room temperature (15–25°C). HiSpeed Plasmid Mega/Giga EF Kits should be stored at room temperature. After adding the RNase A, the Buffer P1 should be stored at 2–8°C and is stable for 6 months. Other buffers and RNase A stock solution can be stored for 2 years at room temperature. The QIAvac HiSpeed LS should be stored clean at room temperature.

Further information

- *HiSpeed Plasmid Mega/Giga EndoFree Purification Handbook:* www.qiagen.com/HB-0185
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: **support.qiagen.com**

Notes before starting

- Assemble the QIAvac HiSpeed LS with vacuum pump and waste containers.
- Add RNase A solution to Buffer P1, mix, and store at 2–8°C.
- Pre-chill Buffer P3; add LyseBlue® reagent to Buffer P1.
- Add 40 mL ethanol (96–100%) to endotoxin-free water supplied with the kit.
- Symbols: ▲values for Mega EF Kit; ● values for Giga EF Kit.
- See Table 1 for volume of buffers required and vacuum pressures used.
- Make sure that the QIAGEN-tips do not dry out during purification.

Procedure

1. Inoculate ▲ 500 mL or ● 2.5 liters medium. Grow at 37°C for 12–16 hours with shaking (300 rpm). Centrifuge at 6000 x *g* for 15 minutes at 4°C.
2. Resuspend the bacterial pellet in Buffer P1. Add Buffer P2, mix thoroughly, and incubate at room temperature for 5 minutes. Add Buffer P3 and mix.
3. Pour lysate into the QIAfilter Mega-Giga Cartridge. Incubate at room temperature for 10 minutes. Draw lysate through the cartridge by vacuum.
4. Add Buffer FWB2 to the cartridge. Draw the liquid through the cartridge by vacuum. Add ▲ 18 mL or ● 40 mL Buffer ER (approx. 10% of the filtrated lysate volume) to the filtered lysate, mix by inverting the bottle approximately 10 times.
5. Equilibrate the required number of QIAGEN-tips by applying Buffer QBT.
6. Draw the lysate through the QIAGEN-tips by vacuum. Wash the QIAGEN-tips with Buffer QC.
7. Elute the plasmid DNA from the QIAGEN-tip with Buffer QN.
8. Precipitate the plasmid DNA by adding ▲ 28 mL or ● 77 mL room-temperature isopropanol to the eluted plasmid DNA in the collection vessel.

9. Attach the Tube Extender to the QIAconcentrator. Transfer the eluate/isopropanol mixture into the empty Tube Extender.
10. Draw the solution completely through the QIAconcentrator, then wash the DNA by adding 10 mL 70% ethanol to the QIAconcentrator.
11. Transfer the QIAconcentrator into a 50 mL collection tube. Centrifuge at 4500–5000 x *g* for 5 minutes (swing out bucket). Place the QIAconcentrator in a new 50 mL collection tube.
12. Add ▲ 1 mL or ● 5 mL Buffer TE to the QIAconcentrator. Stand for 1 min. Centrifuge at 4500–5000 x *g* for 3 minutes at room temperature.

Table 1. Volume of buffers required and vacuum pressures used

		Volume of buffer		
Action (Protocol Step)	Buffer	▲ Mega (mL)	● Giga (mL)	Vacuum Pressure
Resuspend pellet (step 2)	P1	50	125	–
Lysis (step 2)	P2	50	125	–
Neutralization (step 2)	P3	50	125	–
Wash cartridge (step 4)	FWB2	50	50	–
Prepare lysate (step 4)	ER	12.5	30	–
Equilibrate QIAGEN-tip (step 5)	QBT	35	75	Up to –100 mbar
Load QIAGEN-tip (step 6)	–	–	–	Up to –300 mbar
Wash QIAGEN-tip (step 6)	QC	150	300	Up to –500 mbar
Elution from QIAGEN-tip (step 7)	QN	35	100	Up to –200 mbar
Load/wash QIAconcentrator (step 10)	–	–	–	Up to –200 mbar

Document Revision History

Date	Changes
03/2012	Initial release.
12/2023	Revised procedure step 4.

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