EZ1® ccfDNA Midi Kit

Store Magnetic Bead Suspension at 2–8°C upon arrival. Store all other kit components dry at RT.

Further information

- *EZ1 ccfDNA Handbook:* **www.qiagen.com/HB-2346**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **www.support.qiagen.com**

Notes before starting

- Ensure a thermal mixer is RT for use in step 4. Ensure availability of magnetic racks for 15 ml and 2 ml tubes.
- If the lysis buffer in the reagent cartridge forms a precipitate during storage, redissolve at 37°C, then place at RT; do not use damaged cartridges.
- Resuspend Magnetic Bead Suspension by pulse-vortexing for 1 min. Do not let the suspension settle for more than 2 min. Pipet from the center of the suspension.
- Before loading reagent cartridges into the EZ1 instrument, invert them three times to mix the magnetic particles, and then tap to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

Procedure

1. Mix components (see table below) in a 15 ml tube; incubate 10 min at RT while slowly shaking end-over-end.

Plasma (ml)	Magnetic Bead Suspension (µl)	Proteinase K (µl)	Bead Binding Buffer (µl)
4	120	220	600
5	150	275	750
6	180	330	900
7	210	385	1050
8	240	440	1200
9	270	495	1350
10	300	550	1500

2. Spin briefly (30 s at 200 x *g*) to remove any solution in cap. Place the tube with the bead solution on the magnetic rack. Incubate at least 1 min, until the solution is clear. Discard the supernatant.
3. Remove the tube from the magnetic rack. Add 200 µl Bead Elution Buffer to the bead pellet; vortex. Pipet up and down to mix and rinse the tube wall. Transfer bead mixture into Bead Elution Tube (provided). Incubate 5 min on thermal mixer at RT, 300 rpm.
4. Place the Bead Elution Tube with the bead solution on the magnetic rack. Incubate at least 1 min. Transfer supernatant (pre-eluate) to new 1.5 ml elution tube for use in step 7.

   Note: Each sample requires two 1.5 ml elution tubes: one for loading the pre-eluate on the EZ1, and one to collect the ccfDNA after purification on the EZ1. The worktable setup in step 7 will guide you.
5. Insert the EZ1 Advanced XL ccfDNA Mini/Midi Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL instrument; switch on the EZ1 instrument.
6. Press **START** to start the worktable setup of the EZ1 ccfDNA Midi protocol; open the instrument door. Follow the onscreen instructions for the worktable setup and data tracking. Close the instrument door; press **START** to start the protocol.
7. The display will show "Protocol finished" when finished. Press **ESC**.
8. Open the instrument door. Remove the elution tubes containing the purified ccfDNA (in 60 µl) from the first row. Discard the sample preparation waste.* Press **ENT**. The report file is transferred automatically.

   Optional: Follow the onscreen instructions for UV decontamination of worktable surfaces.
9. Perform regular maintenance after each run. Press **ESC** to return to the Main Menu.

* Sample waste contains guanidine salts and is not compatible with bleach.

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