Maxwell® RSC ccfDNA LV Plasma Kit

Instructions for Use of Product AS1840.

Quick Protocol

This Quick Protocol provides instructions for use of the Maxwell® RSC ccfDNA LV Plasma Kit (Cat.# AS1840) with Maxwell® Instruments to purify circulating cell-free DNA (ccfDNA) from plasma samples. For detailed instructions, including information on instrument setup and troubleshooting, please refer to the *Maxwell® RSC ccfDNA LV Plasma Kit Technical Manual* #TM673, available at: **www.promega.com/protocols/**

Preparing Plasma Samples

Materials to Be Supplied by the User

- whole blood or plasma
- benchtop centrifuge

Whole blood should be processed immediately after collection or stored at +2°C to +10°C until plasma preparation. Centrifuge whole blood from EDTA tubes for 10 minutes at 2,000 × *g* to pellet the red and white blood cells. For stabilized blood collection tubes, follow the manufacturer's instructions.

After either EDTA or stabilized blood collection tubes are first centrifuged, use a pipette to carefully remove as much plasma as possible without disturbing the buffy coat. To ensure that no white blood cells are transferred, centrifuge the plasma a second time for 10 minutes at 2,000 × *g*, and transfer the supernatant to a clean tube.

Store plasma at +2°C to +10°C for up to 1 week. For longer storage times, store plasma at –10˚C to –30˚C (or below –65˚C). Avoid exposing plasma to freeze-thaw cycles.

Manual Sample Preprocessing Using a Rotisserie Shaker

1. Add 2–8ml of plasma to a 15ml or 50ml tube. Add an equal volume of Binding Buffer.
2. Shake the bottle containing the Maxwell® Resin E until it is **completely** resuspended.
3. Add 100µl of magnetic resin.
4. Incubate for 45 minutes while shaking. We recommend a rotisserie shaker; the resin must be kept in suspension for the entire incubation.
5. Centrifuge the tubes at 1,000 × *g* for 2 minutes to pellet the resin. Alternatively, a magnetic stand can be used to immobilize the resin.
6. Carefully decant the supernatant. While decanting, we recommend placing a magnet alongside the resin pellet in the tube to fix it in place. Proceed to the section Automated ccfDNA Purification.

Preprocessing Samples with Heater Shaker Magnet Instrument (HSM 2.0)*

1. Add 2–8ml of plasma to a 50ml tube. Add an equal volume of Binding Buffer.
2. Shake the bottle containing the Maxwell® Resin E until it is completely resuspended.
3. Add 100µl of magnetic resin to each tube.
4. Place the tube(s) in the HSM.
5. Open the Promega HSM 2.0 Application Software and select **Start Protocol**.
6. A window will open to select the method. Double-click the "HSM 2.0 RSC ccfDNA LV Plasma v1.0.0.nsp" tile or select the file and choose **Open** to launch the method.
7. The 'Select Available HSM 2.0 Instrument' window will open. Select the HSM 2.0 unit that will run the method and choose **OK**.
8. The protocol window will launch. Press **Start** and follow the instructions in the software.
9. The HSM will shake for 45 minutes and then stop. The magnets will engage, drawing the resin to the side of the tubes.
10. When the resin is completely magnetized, use a pipette to remove the supernatant. Remove the tubes from the HSM. Proceed to the section Automated ccfDNA Purification.

*HSM 2.0 Instrument (Cat.# A2715) must be purchased separately.

Additional protocol information in Technical Manual #TM673, available online at: **www.promega.com**

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Automated ccfDNA Purification

Preparing the Cartridge

1. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
2. Using a pipette, transfer 500µl of well #1 (the large well) into the tube containing the magnetic resin pellet.
3. Resuspend the resin in the reduced volume. We recommend using a pipette for resuspending the resin because vortexing might cause resin to adhere to the upper sides of the tube.

Figure 1. Setup and configuration of the deck tray. The CSC/RSC plunger is placed in well #8 of the cartridge (the well closest to the Elution Tube), and lysates and Lysis Buffer are placed into well #1 of the cartridge.

4. Transfer the resin and liquid back to well #1 of each cartridge (well #1 is the largest well).
5. Place one plunger into well #8 of each cartridge.
6. Place an empty 0.5ml Elution Tube into the elution tube position for each cartridge in the deck tray. Add 75µl of the appropriate elution buffer to the bottom of each elution tube (see Notes below). This will give a final elution volume of approximately 60µl after processing.

   Notes:

   a. We have developed two optimized elution buffer formulations for this kit. The NGS Elution Buffer (Cat.# MC1521) will elute ccfDNA that is predominantly double-stranded. The PCR Elution Buffer (Cat.# MC1511) gives the most efficient elution that works well in amplification-based assays. Ensure the selected elution buffer is at the bottom of the tube for optimal elution.

   b. The NGS Elution Buffer is optimized for assays that require dsDNA. These assays include fluorescent dye quantitation, electrophoresis and whole genome sequencing.

   c. The PCR Elution Buffer is optimized for assays that use both ssDNA and dsDNA. These assays include quantitative PCR, droplet digital PCR and amplicon-based sequencing.

7. Proceed to the next section, Running the Method on Maxwell® Instruments.

Running the Method on the Maxwell® Instruments (Cat.# AS4500, AS6000*, AS8500, AS8000*)

1. To run the Maxwell RSC ccfDNA LV Plasma method, the appropriate Maxwell® RSC ccfDNA LV Plasma method must installed on your instrument. Methods are available at: **www.promega.com/resources/software-firmware/**
2. Follow the instrument run instructions in the *Maxwell® RSC ccfDNA LV Plasma Kit Technical Manual* #TM673.

*The Maxwell® RSC ccfDNA LV Plasma Kit is compatible with Maxwell® CSC Instruments operating in RUO mode.

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