Preparing Plasma Samples for ccfDNA Purification

This Quick Protocol provides instructions for use of the Maxwell® RSC ccfDNA Plasma Kit with the Maxwell® RSC Instrument to purify circulating cell-free DNA (ccfDNA) from human plasma samples. For detailed instructions, including information on instrument setup and troubleshooting, please refer to the Maxwell® RSC ccfDNA Plasma Kit Technical Manual #TM454, available at: www.promega.com/protocols/

Materials to Be Supplied by the User

- whole blood or plasma
- table-top centrifuge (optional)

Preparing the Plasma

Centrifuge whole blood from EDTA tubes for 10 minutes at 2,000 × g to pellet red and white blood cells. Using a pipettor, carefully remove as much plasma as possible without disturbing the pellet. Centrifuge the plasma a second time for 10 minutes at 2,000 × g and transfer the supernatant to a clean tube.

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

Automated DNA Purification

Preparing the Cartridge

1. Place each cartridge to be used in the Maxwell® RSC Deck Tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tube.

2. Press down on the cartridge to snap it into position. Ensure both cartridge ends are fully seated in the deck tray. Carefully peel back the seal so that all plastic comes off the top of the cartridge.

   Note: If you are processing fewer than 16 samples, center the cartridges on the cartridge rack.

3. Place one CSC/RSC Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube. Note: Use only the plungers provided in the Maxwell® RSC ccfDNA Plasma Kit.

4. Place an empty 0.5ml Elution Tube into the elution tube position for each cartridge in the deck tray. Add 60µl of Elution Buffer to the bottom of each Elution Tube.

   Note: Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® RSC Instrument.

5. Add 0.2–1.0ml of plasma to the Binding Buffer in well #1. Mixing is not required.

   Note: Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe, then water. Do not use bleach on any instrument parts.

Figure 1. Setup and configuration of the deck tray. Elution Buffer is added to the elution tubes. Plungers are in well #8 of the cartridge. Plasma sample is added to well #1 of the cartridge.

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Running the Method on the Maxwell® RSC Instrument (Cat.# AS4500)

1. Refer to the Maxwell® RSC Instrument Operating Manual #TM411 for detailed information on running methods. To run the RSC ccfDNA Plasma protocol, the Maxwell® RSC ccfDNA Plasma method must be installed on your instrument. The method is available at: www.promega.com/resources/tools/maxwellrscmethod/. See the Maxwell® RSC Methods Installation Technical Manual #TM435 for instructions.

2. Follow the instrument run instructions in the Maxwell® RSC ccfDNA Plasma Kit Technical Manual #TM454.

Additional protocol information in Technical Manual #TM454, available online at: www.promega.com