

Experimental Protocol

cfDNA extraction using the Maxwell system (Promega)

Total time for experiment: 70 minutes

Reagents:

Maxwell® RSC ccfDNA Plasma Kit

Instruments and tools:

p20, p200 and p1000 pipettes (e.g. Gilson)

Prior considerations:

This protocol can be used to process 0.2ml to 1ml of plasma.

The Maxwell® RSC Instrument can process up to 16 samples.

The hands-on time for this protocol is 10-20 minutes as this is an automated system.

Steps:

Maxwell® RSC ccfDNA Plasma Cartridge Preparation (hands-on):

- Place the cartridges to be used 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.
- Transfer 0.2–1.0ml of each plasma sample to well #1 of each cartridge (well #1 is the largest well) and mix by pipetting up and down to ensure all plasma has been transferred. *N.B. Change pipette tips between samples.*
- Place one plunger into well #8 of each cartridge.
- Place an empty 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. *N.B. This will give a final elution volume after processing of approximately 50µl as typically, the resulting elution volume will be approximately 10µl less than the starting volume.*

Maxwell® Instrument Setup and Run (Instrument set-up):

- Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC and start the Maxwell® software by double-touching the icon on the desktop. The instrument will power up, proceed through a self-test and home all moving parts.
- Perform a contamination of the equipment by clicking the “sanitize” tab.
- Touch Start to access the ‘Methods’ screen and select the cfDNA Plasma method and press the Proceed button.
- On the ‘Cartridge Setup’ screen (if shown), touch the cartridge positions to select/deselect any positions to be used for this extraction run. Enter any required sample tracking information, and touch the “Proceed” button to continue.
- After the door has been opened, transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® instrument platform and confirm that all extraction checklist items have been performed:
 - Plasma samples were added to well #1 of the cartridge
 - Cartridges are loaded on the instrument
 - Uncapped elution tubes are present and Elution Buffer has been added
 - Plungers are in well #8.
- The protocol will take around 1 hour to run. When finished remove the tray from the machine and retrieve the elution tubes with the isolated cfDNA.

N.B. If plasma isolation of more than 1ml of plasma is required, the extraction can be repeated with an additional 1ml of plasma using the original eluted cfDNA sample instead of elution buffer in order to obtain a more concentrated sample.

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