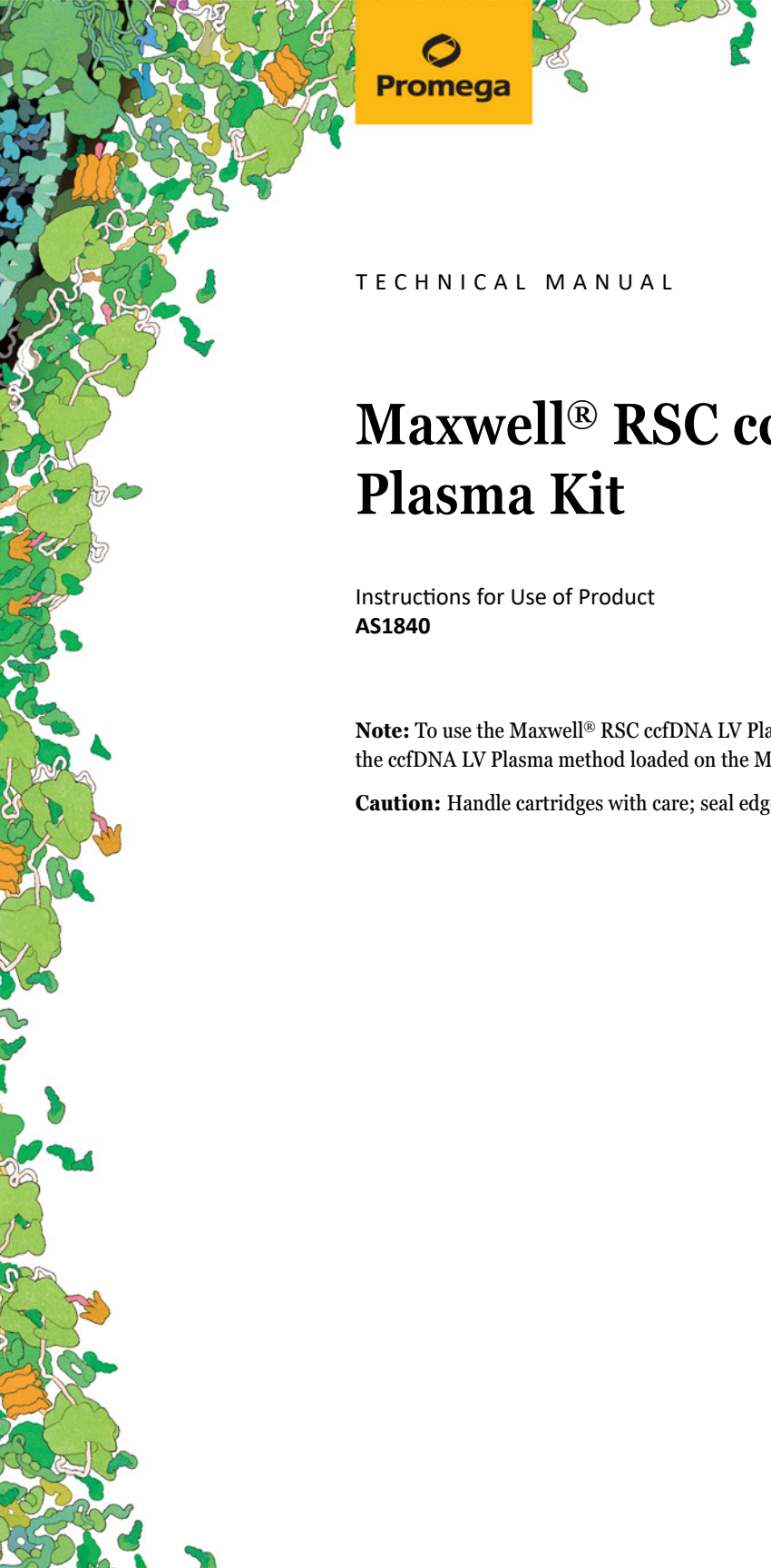

TECHNICAL MANUAL

Maxwell® RSC ccfDNA LV Plasma Kit

Instructions for Use of Product
AS1840

Note: To use the Maxwell® RSC ccfDNA LV Plasma Kit, you must have the ccfDNA LV Plasma method loaded on the Maxwell® Instrument.

Caution: Handle cartridges with care; seal edges may be sharp.

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Maxwell® RSC ccfDNA LV Plasma Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Maxwell® RSC ccfDNA LV Plasma Kit[a,b] is used with Maxwell® Instruments and the HSM 2.0 Instrument or a rotisserie mixer as specified below to provide an easy method for efficient, semi-automated sample preparation and purification of circulating cell-free DNA (ccfDNA) from human plasma samples. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC ccfDNA Plasma Kit can process from one to the maximum number of samples allowed in approximately 90 minutes. The kit contains two optimized DNA elution buffers to support PCR and next-generation sequencing (NGS) applications.

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1. Description (continued)

Table 1. Supported Instruments

Instrument	Cat.#	Technical Manual	Maximum Sample Number
Maxwell® RSC	AS4500	TM411	16
Maxwell® RSC 48	AS8500	TM510	48
Maxwell® FSC	AS4600	TM462	16
Maxwell® CSC RUO Mode	AS6000	TM573	16
Maxwell® CSC 48 RUO Mode	AS8000	TM628	48
HSM 2.0	A2715	TM389	32

The Maxwell® RSC ccfDNA Plasma Kit purifies circulating cell-free DNA (ccfDNA) using a novel paramagnetic particle that provides a mobile solid phase to optimize sample capture, washing and purification of circulating DNA. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind circulating DNA to the paramagnetic particle in the first well of a prefilled cartridge and mix during processing.

Prior to extraction, samples can be preprocessed manually using a rotisserie or the Heater Shaker Magnet (HSM 2.0) Instrument. The HSM is used for the lysis, binding, and magnetic capture of ccfDNA bound to the paramagnetic particles. Follow the instructions specific to the preprocessing option used.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
Maxwell® RSC ccfDNA LV Plasma Kit	**48 preps**	**AS1840**

For Research Use Only. Sufficient for 48 automated isolations from 2–4ml of plasma samples. Includes:

- 48 Maxwell® RSC Cartridges (Note: No paramagnetic particles are predispensed)
- 200ml Binding Buffer (BBC)
- 10ml Maxwell® Resin E
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 5ml PCR Elution Buffer (EBE)
- 5ml NGS Elution Buffer (EBF)

Additional Binding Buffer (BBC) can be purchased separately (Cat.# MC1361) for processing plasma volumes 4–8ml.

Storage Conditions: Store the Maxwell® RSC ccfDNA LV Plasma Kit at +15°C to +30°C.

Safety Information: The Maxwell® RSC LV Cartridges contain ethanol, isopropanol, guanidine thiocyanate and guanidine hydrochloride. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine thiocyanate and guanidine hydrochloride should be considered toxic, harmful and irritants. Refer to the SDS for detailed safety information.

Maxwell® RSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances used with this system.

Caution: Handle cartridges with care; seal edges may be sharp. Bleach reacts with guanidine thiocyanate and should not be added to any sample waste from these cartridges.

3. Usage Information

3.A. Intended Use

The Maxwell® RSC ccfDNA LV Plasma Kit is intended for use in combination with Maxwell® Instruments and the Maxwell® ccfDNA Plasma purification method, and is for research use only. The kit is intended for blood samples collected in EDTA or stabilized blood collection tubes.

3.B. Limitations of Use

The Maxwell® RSC ccfDNA LV Plasma Kit is only intended for use with plasma prepared from human whole blood samples collected in EDTA or stabilized blood collection tubes. It is not intended for use with whole blood, bone marrow or buffy coat samples, or samples stored in other collection tubes or samples stored outside of the product claims. The Maxwell® RSC ccfDNA LV Plasma Kit is not intended for use in diagnostic procedures.

4. 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 2–10°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 ccfDNA stabilizer tubes, follow 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 one week. For longer storage times, store plasma at –10˚C to –30˚C (or below –65˚C). Avoid exposing plasma to freeze-thaw cycles.

5. 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 prior to decanting.
6. Carefully decant the supernatant. While decanting, we recommend placing a magnet alongside the resin pellet in the tube to hold it in place. Proceed to Section 7, Preparing Maxwell® RSC ccfDNA LV Plasma Cartridges.

6. Preprocessing Samples with Heater Shaker Magnet Instrument (HSM 2.0)

For information and instructions on HSM 2.0 setup and operation, see the *HSM 2.0 Instrument Technical Manual* #TM389, available at: **www.promega.com/protocols**

Notes:

a. The HSM 2.0 RSC ccfDNA LV Plasma method is available for download at: **www.promega.com/resources/software-firmware/other/hsm-2-0-software/**

b. Install the HSM 2.0 method by saving the method on the computer that operates the HSM 2.0 instrument in the following directory: C:\ProgramData\Promega\HSMv2\Data\Protocols

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. Once the resin is completely magnetized, use a pipette to remove the supernatant. Remove the tubes from the HSM. Proceed to Section 7, Preparing Maxwell® RSC ccfDNA LV Plasma Cartridges.

7. Preparing Maxwell® RSC ccfDNA LV Plasma Cartridges

1. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). 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.
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.
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 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. 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 will elute ccfDNA that is predominantly double-stranded. The PCR Elution Buffer gives the most efficient elution that works well in amplification-based assays.

   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 utilize both ssDNA and dsDNA. These assays include quantitative PCR, droplet digital PCR and amplicon-based sequencing.
7. Proceed to Section 8, Maxwell® Instrument Setup and Run.

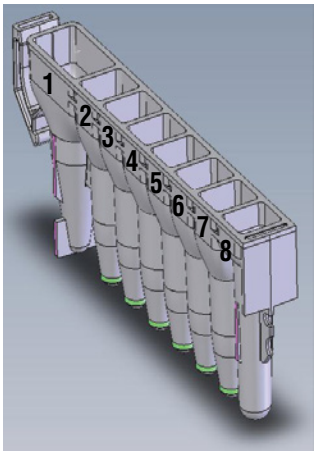
   Notes:

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

   b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.

   c. The starting volume of elution buffer will not result in the same elution volume after running the method. Typically, the resulting elution volume will be approximately 10µl less than the starting volume.

7. Preparing Maxwell® RSC ccfDNA LV Plasma Cartridges (continued)



User Adds to Wells:

1. 0.5–1.0ml of resin and liquid
8. RSC Plunger

Figure 1. Maxwell® RSC Cartridge.

Figure 2. Setup and configuration of the deck tray(s). Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

8. Maxwell® Instrument Setup and Run

For detailed information, refer to the Technical Manual specific to your Maxwell® Instrument (see Table 1).

1. 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.
2. Touch **Start** to begin the process of running a method.
3. Depending on your Maxwell® Instrument model, use one of the following options to select a method:
   a. When running in Portal mode, scan the bar codes(s) on the deck tray(s). After data has been returned from the Portal database, press **Continue** to use the sample tracking information for the deck tray(s) or press **New** to start a run and enter new sample tracking information.
   b. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method.
   c. Touch the ccfDNA LV Plasma method.

Figure 3. Kit label indicating the bar code to scan. The bar code to scan for starting a purification run is shown in the red box, in the upper right of the kit label.

4. If applicable to your Maxwell® Instrument model, verify that the ccfDNA Plasma method has been selected, and press the **Proceed** button. If requested by the software, scan or enter any kit lot and expiration information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select or deselect the positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using 48-position Maxwell® Instruments, press the **Front** and **Back** buttons to select or deselect cartridge positions on each deck tray.

8. Maxwell® Instrument Setup and Run (continued)

6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® instrument platform.

   Inserting the Maxwell® deck tray(s): Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

   Note: Check the identifier on 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument.

7. Touch the **Start** button to begin the extraction run. The platform will retract, and the door will close.

   Note: If the Vision System has been enabled when using a 48-position Maxwell® Instrument, the deck trays will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen and problem positions will be marked with an exclamation point in a red circle. Touch the exclamation point for a description of the error and resolve all error states. Touch the **Start** button again to repeat deck tray scanning and begin the extraction run.

   Warning: Pinch point hazard.

   The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being performed and the approximate time remaining in the run.

   Notes:

   1. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
   2. If a run is abandoned before completion, you may be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up**. The samples will be lost.

8. Follow on-screen instructions at the end of the method to open the door. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the Technical Manual appropriate to your Maxwell®Instrument (see table above) to perform a **Clean Up** process to attempt to unload the plungers.

9. Remove the deck tray(s) from the instrument. Remove elution tubes containing ccfDNA and cap the tubes. After the run has been completed, the extraction run report will be displayed. From the report screen, you can print or export this report or both.

10. Remove the cartridges and plungers from the deck tray, and discard as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

Ensure samples are removed before performing any required UV light treatment to avoid damage to the nucleic acid.

9. Considerations When Working with ccfDNA

9.A. Preparing Plasma

One potential issue when purifying ccfDNA is the presence of contaminating genomic DNA from lysed white blood cells. Plasma is typically centrifuged twice; the first spin removes the red and white blood cells, and the second spin removes any residual white blood cells. If the blood sample was incubated for extended periods at room temperature, or was frozen and thawed prior to processing, some white blood cells may have lysed, releasing genomic DNA into the plasma.

When using ccfDNA stabilized blood collection tubes, follow manufacturer's instructions prior to processing. If the manufacturer suggests using Proteinase K, it should be added to the sample prior to performing the lysis/binding incubation.

If the plasma sample has been frozen, cryoprecipitate might be present after thawing. While cryoprecipitate has no effect on the purification of ccfDNA with the Maxwell® RSC ccfDNA Plasma Kit, it can affect plasma pipetting. To pellet the cryoprecipitate, centrifuge the plasma sample at 1,000 × *g* for 5 minutes prior to processing.

9.B. Recommendations for Quantitating ccfDNA

The low concentration and fragmented nature of ccfDNA provide unique challenges for researchers. In normal plasma, yields of 5–20ng of ccfDNA per milliliter of plasma are typical. The majority of ccfDNA fragments are approximately 170bp, with additional fragments at approximately 340bp and 510bp.

Quantitating by UV

It is impossible to get an accurate determination of ccfDNA concentration using 260nm absorbances due to the low concentration. Some available products use a carrier RNA to facilitate purification of ccfDNA. The carrier RNA is in much higher abundance than the ccfDNA and copurifies. This can give a false A_{260} value and drastically higher apparent ccfDNA concentrations. For accurate quantitation, use fluorescent dyes or PCR.

Quantitating by Fluorescence

NGS elution buffer must be used if quantitating by fluorescent dye. This elution buffer helps to maintain dsDNA, which is important when using fluorescent dyes that intercalate.

The sensitivity of dsDNA-specific dyes makes them a better choice for quantitating ccfDNA, but there are two concerns. The first involves carrier RNA. While dsDNA-specific dyes have a much higher specificity for DNA than RNA, the high levels of carrier RNA in other ccfDNA kits inflate the RFU values, making ccfDNA levels appear higher than actual concentrations.

A second factor is that the standards used in fluorescent dyes are typically high-molecular-weight genomic or lambda DNA. ccfDNA is highly fragmented and does not bind fluorescent dyes as effectively as high-molecular-weight DNA, leading to lower apparent concentrations. If possible, use lower molecular weight DNA standards for more accurate quantitation.

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Quantitating by PCR

Use PCR elution buffer when quantitating by amplification. This elution buffer efficiently elutes ssDNA as well as dsDNA, which can both be amplified in qPCR, ddPCR or amplicon-based sequencing.

Quantitating with either qPCR or digital droplet PCR gives the most accurate measure of ccfDNA. In addition to sensitivity, amplification-based quantitation can indicate suitability of samples for amplification-based downstream applications.

10. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Instrument unable to pick up plungers	Make sure you are using an RSC-specific chemistry kit; the plungers for the Maxwell® RSC reagent kits are specific for supported Maxwell® Instruments (See Section 1).
	Make sure to use plungers from the Maxwell® RSC Plunger Rack.
Low yield	In normal plasma, yields of 2–20ng of ccfDNA per milliliter of plasma are typical. The Maxwell® RSC ccfDNA LV Plasma kit can accept up to 8ml plasma sample.
Low yield with ccfDNA stabilized blood collection tubes	Confirm Proteinase K addition per manufacturer's recommendations.
Low yield by fluorescent dye	Confirm that the NGS elution buffer was used.
Low yield by amplification	Confirm that the PCR elution buffer was used.
Wrong method run on the Maxwell® Instrument	Check that the ccfDNA LV Plasma method was run.
Ethanol contamination affects downstream applications	Excess ethanol in eluate. To remove the contaminating ethanol, uncap Elution Tube and and place in 60° heat block for 30 minutes to evaporate.

11. Related Products

Instruments

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® CSC 48 Instrument	1 each	AS8000
Maxwell® RSC Plunger Pack	1 each	AS1670
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® FSC Deck Tray	1 each	AS4016
Maxwell® RSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC 48 Back Deck Tray	1 each	AS8402
Heater Shaker Magnet Instrument (HSM 2.0)	1 each	A2715

Accessories

Product	Size	Cat.#
Nunc™ 2.0ml Deep Well Plates	60/pack	AS9307
Binding Buffer (BBC)	200ml	MC1361
PCR Elution Buffer	5ml	MC1511
NGS Elution Buffer	5ml	MC1521
Elution Tubes	50/pk	AS6201

Maxwell® RSC Reagent Kits

For a list of available Maxwell® RSC purification kits, visit: **www.promega.com**

[a]U.S. Pat. No. 6,855,499 and other patents.

[b]U.S. Pat. No. 7,329,488 and S. Korean Pat. No. 100483684.