TECHNICAL MANUAL

Maxwell® CSC RNA FFPE Kit

Instructions for Use of Product
AS1360

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

INSTRUCTIONS FOR USE OF PRODUCT
AS1360

IVD

Revised 8/19
TM404

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Maxwell® CSC RNA FFPE 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

The Maxwell® CSC RNA FFPE Kit is only available in certain countries. This product meets the essential requirements of EU Directive 98/79/EC on in vitro diagnostic medical devices.

1. Description

The Maxwell® CSC RNA FFPE Kit is used in combination with the Maxwell® CSC Instrument to provide an easy method for efficient, automated purification of RNA from human FFPE (formalin-fixed, paraffin-embedded) breast, lung or colon tissue samples. The Maxwell® CSC Instrument is supplied with preprogrammed purification methods. It is designed for use with the predispensed reagent cartridges and additional reagents supplied in the kit, thereby maximizing simplicity and convenience. The instrument can process up to 16 samples in less than 60 minutes, and the purified RNA can be used directly in a variety of amplification-based downstream applications such as RT-PCR.

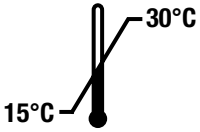
1. Description (continued)

The Maxwell® CSC RNA FFPE Kit purifies nucleic acid using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of RNA. The Maxwell® CSC Instrument is a magnetic particle-handling instrument. This system allows efficient binding of RNA to the paramagnetic particles in the first well of a prefilled cartridge and moves the sample through the wells of the cartridge, mixing during processing. This approach to magnetic capture avoids common problems associated with liquid-handling systems such as clogged tips or partial reagent transfers, which result in suboptimal purification processing by other commonly used automated systems.

2. Product Components, Storage Conditions and Symbols Key

PRODUCT	SIZE	CAT.#
Maxwell® CSC RNA FFPE Kit	**48 preps**	**AS1360**

For In Vitro Diagnostic Use. Professional use only. Sufficient for 48 automated isolations from FFPE samples. The Maxwell® CSC Cartridges are for single use only.



Includes:

- 25ml Mineral Oil
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K
- 100µl Blue Dye
- 2 × 1ml $MnCl_2$, 0.09M
- 1ml DNase Buffer
- 3 vials DNase I (lyophilized)
- 48 Maxwell® FFPE Cartridges
- 50 CSC/RSC Plungers
- 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

Storage Conditions: Store the Maxwell® CSC RNA FFPE Kit at ambient temperature (15–30°C). Store rehydrated DNase I at –10 to –30°C. Do not freeze-thaw more than 10 times.

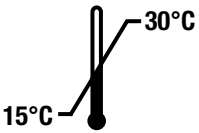
Safety Information: The reagent cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants.

The Maxwell® CSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (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.

Additional Information: The Maxwell® CSC RNA FFPE Kit components are qualified and quality control tested to work together. It is not recommended to mix kit components between different kit lots. Use only the components provided in the kit.

Symbols Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Authorized Representative
	Store at 15–30°C.		Manufacturer
	Caution		Irritant
	Carcinogen		Contains sufficient for "n" tests
	Conformité Européenne		Warning. Biohazard.
	Warning. Pinch point hazard.		Catalog number
	Lot number		Do not reuse

3. Product Intended Use

The Maxwell® CSC RNA FFPE Kit is intended for use, in combination with the Maxwell® CSC Instrument and the Maxwell® CSC RNA FFPE purification method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of RNA from human breast, lung and colon FFPE (formalin-fixed, paraffin-embedded) tissue samples. The purified RNA is suitable for use in amplification-based in vitro diagnostic assays.

The Maxwell® CSC RNA FFPE kit is intended to be used at a temperature between 15°C and 30°C. Use outside of this temperature range may result in suboptimal results.

FFPE samples prepared using 10% neutral-buffered formalin can be used with the Maxwell® CSC RNA FFPE Kit.

The Maxwell® CSC RNA FFPE Kit is not intended for use as part of a specific diagnostic test.

The Maxwell® CSC RNA FFPE Kit is intended for professional use only. Diagnostic results obtained using the RNA purified with this system must be interpreted in conjunction with other clinical or laboratory data.

4. Product Use Limitations

The Maxwell® CSC RNA FFPE Kit is only intended for use with FFPE tissue samples collected from human breast, lung or colon. It not intended for use with non-FFPE tissue samples, such as fresh or frozen tissue samples, or with FFPE tissue samples collected from tissues other than human breast, lung or colon. The Maxwell® CSC RNA FFPE Kit is not intended for use with other types of samples, including non-human samples, or for the purification of DNA.

The Maxwell® CSC RNA FFPE Kit is not intended for use with tissue samples that have been prepared with fixatives other than 10% neutral-buffered formalin.

The Maxwell® CSC RNA FFPE Kit performance has been evaluated by isolating RNA from FFPE tissue samples ranging in size from 0.1–2.0mm^3. It is not intended for use with samples outside this range.

The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications. Appropriate controls must be included in any downstream diagnostic applications using RNA purified using the Maxwell® CSC RNA FFPE Kit.

5. Before You Begin

Materials to be Supplied by the User

- microcentrifuge
- pipettors and pipette tips for sample preprocessing and transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., Microtubes, 1.5ml; Cat.# V1231)
- heat blocks set at 56°C and at 80°C (**Note:** The heat block should be set to the needed temperature. Actual heat block temperature should be measured at temperature within the calibration specifications of the thermometer used for the measurement.)
- FFPE samples with a total tissue volume of 0.1–2.0mm^3; the thickness of the section should not exceed 5µm (**Note:** Samples should be stored at room temperature [15–30°C].)
- razor blades (**Note:** Use caution when using razor blades to scrape sample from the slide.)

As necessary, reconstitute a lyophilized vial of DNase I with 275µl of Nuclease-Free Water. Invert the vial to rinse DNase I off the underside of the cap and swirl gently to mix; do not vortex.

5.A. Preparation of FFPE Samples

Preprocessing of Section Samples

1. Place the section into a 1.5ml microcentrifuge tube. If you are using slide-mounted tissue sections, scrape the section off of the slide using a clean razor blade.
2. Add 300µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
3. Heat the samples at 80°C for 2 minutes. Place the samples at room temperature while the master mix is prepared.
4. Prepare a master mix of the Lysis Buffer, Proteinase K and Blue Dye as shown below:

Reagent	Amount/Reactions	Reactions (Number to be run +1)	Total
Lysis Buffer	224µl	n+1	224µl × (n+1)
Proteinase K	25µl	n+1	25µl × (n+1)
Blue Dye	1µl	n+1	1µl × (n+1)

5. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.
6. Centrifuge sample tubes at 10,000 × *g* for 20 seconds to separate the layers. If a pellet is present in the aqueous layer (lower, blue layer), gently mix to disperse the pellet. Leave both phases in the tube.
7. Transfer the sample tubes to a 56°C heat block, and incubate for 15 minutes.
8. Transfer the sample tubes to an 80°C heat block, and incubate for 1 hour.
9. Remove the sample tubes from the heat block, and allow the samples to cool to room temperature for 15 minutes. While the samples are cooling, prepare the DNase cocktail as described in Step 10.

5.A. Preparation of FFPE Samples (continued)

10. Prepare a cocktail of $MnCl_2$, DNase buffer and DNase I in the order shown below:

Reagent[1]	Amount/Reactions	Reactions (Number to be run +1)	Total
$MnCl_2$, 0.09M	26µl	n+1	26µl × (n+1)
DNase Buffer[2]	14µl	n+1	14µl × (n+1)
DNase I[3]	10µl	n+1	10µl × (n+1)

[1]If the DNase cocktail reagents are added individually to sample tubes, be certain to add them in the order shown above. Incorporate each reagent by thoroughly pipetting before adding the next reagent.

[2]Store DNase Buffer at 15–30°C; it can precipitate if stored at lower temperatures. If the buffer contains precipitate, resolubilize the precipitate by heating to 56°C for 2 minutes followed by vortexing briefly to mix.

[3]Store remaining reconstituted DNase I at –30 to –10°C.

11. Add 50µl of DNase cocktail to the blue, aqueous phase of each sample tube. Mix by pipetting 10 times.
12. Incubate the sample tubes for 15 minutes at room temperature (15–30°C).
13. Centrifuge the sample tubes at full speed in a microcentrifuge for 5 minutes.
14. Immediately transfer the blue, aqueous phase to well #1 of a Maxwell® CSC Cartridge. See Section 5.B. for cartridge preparation instructions.

5.B. Maxwell® CSC Cartridge Preparation

1. Change gloves before handling Maxwell® CSC Cartridges, CSC/RSC Plungers and Elution Tubes. Cartridges are set up on the Maxwell® CSC Deck Tray outside of the instrument, and the deck tray containing the cartridges and samples is transferred to the instrument for purification. Place cartridges to be used in the Maxwell® CSC Deck Tray (Figure 2). Place each cartridge in the deck tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes. 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 the entire seal is removed from the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed from the cartridge.

Caution: Handle cartridges with care. Seal edges may be sharp.

2. Place one plunger into well #8 of each cartridge.

   Note: Use only the plungers provided in the Maxwell® CSC RNA FFPE Kit. Plungers for the Maxwell® 16 LEV and SEV Kits are not compatible with the Maxwell® CSC Instrument.

3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the Maxwell® CSC Deck Tray.

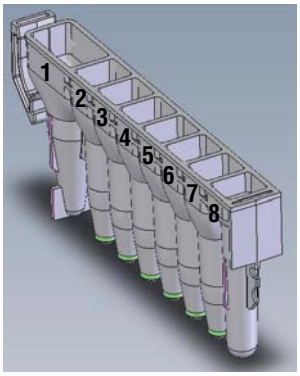
   Note: Use only the elution tubes provided in the Maxwell® CSC RNA FFPE Kit. Other elution tubes may not be compatible with the Maxwell® CSC Instrument and may affect RNA purification performance.

4. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. The elution tubes must remain open during the RNA purification.

   Note: Use only the Nuclease-Free Water provided in the Maxwell® CSC RNA FFPE Kit. Use of other elution buffers may impact RNA purification performance or downstream use.

Maxwell® FFPE Cartridge Preparation Notes

Clean specimen or reagent spills on any part of the Maxwell® CSC Deck Tray as indicated in the *Maxwell® CSC Instrument Operating Manual* #TM457. Do not use bleach on any instrument parts.



Well Content User Adds:
1. Preprocessed samples
8. CSC/RSC Plunger

Figure 1. Maxwell® CSC Cartridge. The preprocessed FFPE sample is added to well #1, and a plunger is added to well #8.

Figure 2. Setup and configuration of the Maxwell® CSC Deck Tray. Nuclease-Free Water is added to the Elution Tubes as indicated.

6. Instrument Run

The Maxwell® CSC RNA FFPE Method can be downloaded from the Promega Web site: **www.promega.com/resources/tools/maxwellcscmethod**. See the *Maxwell® CSC Instrument Operating Manual* #TM457 for more detailed information.

If you suspect your instrument may be contaminated with RNase, clean the instrument prior to running it using a detergent solution such as Steris LpH®. Follow the instructions in the Cleaning and Maintenance section of the *Maxwell® CSC Instrument Operating Manual* #TM457.

1. Turn on the Maxwell® CSC Instrument and Tablet PC. Log into the Tablet PC and start the Maxwell® software by double-touching the Maxwell® CSC IVD icon on the desktop. The instrument will proceed through a self-check and home all moving parts.
2. Select **Start** on the 'Home' screen.
3. Scan or type the method bar code in the upper right corner of the Maxwell® CSC RNA FFPE Kit label to automatically select the method to be run (Figure 3).

   Note: The Maxwell® CSC RNA FFPE Kit method bar code is required for RNA purification on the Maxwell® CSC Instrument. The kit label contains two bar codes. The method bar code is indicated in Figure 3. If the bar code can not be scanned, contact Promega Technical Services.

Figure 3. Kit label indicating the method bar code to scan. Shown in the red box is the method bar code to scan on the kit label for starting a purification run.

4. On the 'Cartridge Setup' screen, touch the cartridge positions to select/deselect any positions to be used for this extraction run. Enter any required sample tracking information and press the **Proceed** button to continue.
5. After the door has opened, confirm that all extraction checklist items have been performed. Verify that preprocessed 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 containing the prepared cartridges to the Maxwell® instrument.

Inserting the Maxwell® deck tray: 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 aginst 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.

Warning: Pinch point hazard.

6. Confirm all the indicated preprocessing has been performed, and touch **Start** to close the instrument door and start processing.

   Note: If the run is aborted before completion, the samples will be lost. Do not attempt to repurify samples for which an instrument run was aborted.

7. When the automated purification run is complete, the user interface will display a message that the method has ended.

End of Run

8. Follow on-screen instructions at the end of the method to open the door. Verify that the plungers are located in well #8 of the cartridge at the end of the run. If the plungers have not all been removed from the plunger bar: Follow the on-screen prompts to perform the Clean Up method for an aborted run; or select the Clean Up method from the 'Settings' screen for a successfully completed run to remove the remaining plungers.

9. Cap and remove the Elution Tubes containing RNA immediately following the run to prevent evaporation of the eluates. Remove the Maxwell® deck tray from the instrument.

   Note: To remove the deck tray from the instrument platform, hold the deck tray by its sides. Ensure the samples are removed from the instrument before running a UV sanitization protocol to avoid damage to the purified nucleic acid. RNA samples may be stored overnight at –30 to –10°C, or at lower than –60°C for longer-term storage.

10. Remove the cartridges and plungers from the Maxwell® CSC Deck Tray, and discard as hazardous waste according to your institution's procedures. Cartridges, plungers and elution tubes are intended for single use. Do not reuse Maxwell® CSC Cartridges, CSC/RSC Plungers or Elution Tubes.

7. Post-Purification

Determine that the purified RNA sample yield and purity meets the input requirements for the downstream diagnostic assay prior to use in that assay. Kit performance was evaluated based on the purification of amplifiable RNA. Other means of quantification, including absorbance or fluorescent dye binding, may not correlate with amplification (1). Absorbance readings for FFPE samples may over-estimate yield; we recommend using more specific methods for determining yield (1).

8. 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	Possible Causes and Comments
Lower than expected concentration of RNA in eluate (A typical FFPE section should yield amplifiable RNA depending on tissue size, cellularity, formalin fixation condition and handling.)	Kit performance has been evaluated by isolating RNA from FFPE tissue samples ranging in size from 0.1mm^3 to 2.0mm^3. It was not designed for samples outside this range. Use sections that will fall within this range.
	The kit was designed for use with FFPE tissue samples collected from human breast, lung or colon. It was not designed for use with non-FFPE tissue samples, such as fresh or frozen tissue samples, or with FFPE tissue samples collected from tissues other than human breast, lung and colon. Incubation times and temperatures were tested for human breast, lung and colon only. Use only human breast, lung, or colon.
	The kit was not designed for use with tissue samples that have been prepared with fixatives other than 10% neutral-buffered formalin. Confirm that an alternative fixative was not used.
	RNases may have been introduced during sample processing or quantitation. See Section 9 for information on creating a ribonuclease-free environment.
	Tissue used was from a stained slide or section. No claims are made for stained slides or sections. Repeat the purification with an unstained slide or section.
	Kit performance was evaluated based upon the purification of amplifiable RNA. Other means of quantitation including absorbance or fluorescent dye binding may not correlate with amplification. Use an amplification quantitation method to assess yield.
Lower than expected quality (The eluate contains highly fragmented RNA or inhibitors of downstream assays.)	Formalin fixation and subsequent crosslink reversal will fragment RNA. If the RNA is fragmented prior to extraction/purification, fragmented RNA will be purified with this kit. Repeat with an adjacent section to assess whether there is a problem with the selected section or with the process.

Symptoms	Possible Causes and Comments
Lower than expected quality (continued)	Some amplification assays are particularly sensitive to the presence of inhibitors. Downstream assay controls should identify the presence of an amplification inhibitor in the eluate. It is the user's responsibility to verify the compatibility of this product with all downstream assays.
DNA present in eluates (The eluates are contaminated with DNA, which may interfere with downstream assays.)	The DNase cocktail added to the sample provides an excess of DNase activity when used with FFPE tissue samples ranging in size from 0.1mm^3 to 2.0mm^3. It was not designed for samples outside this range and may not be optimal. Use sections that will fall within this range.
	Insufficient mixing of the DNase cocktail into the sample during preprocessing can result in incomplete degradation of DNA. Be sure to mix the DNase cocktail thoroughly into the sample.
	If the DNase cocktail components are added to the sample separately, be sure to add them in the order indicated in Section 5.A, Step 10. In addition, be sure to mix each component thoroughly as it is added. Adding components in a different order or mixing incompletely can inactivate DNase.

9. Appendix

9.A. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Take care to avoid introducing RNase activity into your RNA samples during and after isolation. This is especially important if the starting material is only available in a limited quantity. The following notes may help prevent accidental RNase contamination of your samples.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use aseptic technique when handling the reagents supplied with this system. Wear gloves at all times. Change gloves whenever ribonucleases may have been contacted.
2. Whenever possible, use sterile, disposable plasticware for handling RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat non-sterile glassware and plasticware before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA, followed by RNase-free water. Commercially available RNase removal products also may be used, following the manufacturer's instructions.
4. Treat solutions not supplied with the system by adding diethyl pyrocarbonate (DEPC) to 0.1% in a fume hood. Incubate overnight with stirring at room temperature in the hood. Autoclave for 30 minutes to remove any trace of DEPC.

Caution: DEPC is a suspected carcinogen and should only be used in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

Note: For all downstream applications, it is essential that you continue to protect your RNA samples from RNases.

9.B. Reference

1. Bonin, S. *et al.* (2010) Multicentre validation study of nucleic acids extraction from FFPE tissues. *Virchows Arch.* **457**, 309–17.

10. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® CSC Instrument*	1 each	AS6000
Maxwell® RSC/CSC Deck Tray	1 each	SP6019

*For In Vitro Diagnostic Use. This product is only available in certain countries.

Maxwell® CSC Reagent Kits

Visit **www.promega.com** for a list of available Maxwell® CSC purification kits.

11. Summary of Changes

The following changes were made to the 8/19 revision of this document:

1. Updated cartridge information in Figure 1.
2. Updated information about the Maxwell® CSC Instrument run in Section 6.
3. Added Section 10.