TECHNICAL MANUAL

Maxwell® CSC XtractAll FFPE DNA/RNA Kit

Instructions for Use of Product
AS1560

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

Note: The Maxwell® CSC XtractAll FFPE DNA/RNA Kit is only compatible with Maxwell® CSC software version 4.0.0 or greater or Maxwell® CSC 48 software version 4.1.1 or greater.

PROMEGA
2800 Woods Hollow Rd.
Madison, WI USA

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

INSTRUCTIONS FOR USE OF PRODUCT
AS1560

IVD

5/25
TM755

Promega

Maxwell® CSC XtractAll FFPE DNA/RNA Kit

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

The Maxwell® CSC XtractAll FFPE DNA/RNA Kit is only available in certain countries.

1. Description

The Maxwell® CSC XtractAll FFPE DNA/RNA Kit is used in combination with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated extraction of DNA, RNA or total nucleic acid (TNA), or both DNA and RNA sequentially from formalin-fixed, paraffin-embedded (FFPE) tissue samples. The Maxwell® CSC Instruments are designed for use with predispensed reagent cartridges and additional reagents supplied in the kit. The preprogrammed extraction methods maximize simplicity and convenient use of the Maxwell® CSC Instruments. The Maxwell® CSC Instruments can process from one to the maximum number of samples allowed in an efficient manner with automated extraction of DNA, RNA and total nucleic acid (TNA) in approximately 30 minutes and sequential DNA and RNA extractions in less than 1 hour. Extracted DNA, RNA or total nucleic acid can be used directly in downstream amplification-based assays.

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual	Maximum Sample Number
Maxwell® CSC	AS6000	TM457	16
Maxwell® CSC 48	AS8000	TM623	48

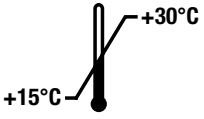
Principle of the Method: The Maxwell® CSC XtractAll FFPE DNA/RNA Kit extracts nucleic acid using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and extraction of DNA, RNA or total nucleic acid. Optionally, both DNA and RNA can be sequentially extracted from the same FFPE tissue sample without the need for splitting the lysate. The Maxwell® CSC Instruments are magnetic particle-handling instruments. This system allows efficient binding of nucleic acid to the paramagnetic particles in the first well of a prefilled cartridge and moves the paramagnetic particles through the wells of the cartridge. This approach to magnetic capture avoids common problems, such as clogged tips or partial reagent transfers, which result in suboptimal extraction processing by other commonly used automated systems.

Sample Considerations: Nucleic acid extraction from FFPE tissue samples can be challenging due to tissue characteristics such as fibrosity, lipid composition, nuclease content and the cell number available in the tissue section. In addition, variability in how the tissue is handled prior to and during fixation, including the duration of exposure to formalin during the tissue fixation process greatly influences the degree of crosslinking and fragmentation of nucleic acid in the FFPE tissue. These attributes can influence the quality and amount of amplifiable nucleic acid that can be extracted from FFPE tissue sections. During development, the Maxwell® CSC XtractAll FFPE DNA/RNA Kit was evaluated with a variety of human FFPE tissue types to extract the available amplifiable DNA, RNA or total nucleic acid.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® CSC XtractAll FFPE DNA/RNA Kit	**48 preps**	**AS1560**

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



Includes:

- 35ml Mineral Oil
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K
- 2 × 100µl Blue Dye
- 2 × 1ml $MnCl_2$, 0.09M
- 3 vials DNase I (lyophilized)
- 48 Maxwell® CSC Cartridges (CSCR)
- 50 CSC/RSC Plungers
- 2 × 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

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

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

The Maxwell® CSC XtractAll FFPE DNA/RNA Kit components are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances used with this system.

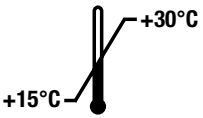
Caution: Handle cartridges with care; seal edges may be sharp.

Promega

2. Product Components and Storage Conditions (continued)

Additional Information: The Maxwell® CSC XtractAll FFPE DNA/RNA Kit components are qualified and quality control tested to work together. Do not mix kit components between different kit lots. Use only the components provided in the kit. Do not use cartridges if the cartridge seal is not intact on receipt.

Symbols Key

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

3. Product Intended Purpose/Intended Use

The Maxwell® CSC XtractAll FFPE DNA/RNA Kit is intended for use, in combination with Maxwell® CSC instruments and the Maxwell® CSC XtractAll methods, as an in vitro diagnostic (IVD) medical device to perform automated isolation of DNA only, RNA only, both DNA and RNA sequentially, or total nucleic acid from human formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The extracted DNA, RNA, or total nucleic acid are suitable for use in amplification-based in vitro diagnostic assays.

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

The Maxwell® CSC XtractAll FFPE DNA/RNA Kit is intended for professional use only. Diagnostic results obtained using DNA, RNA, or total nucleic acid extracted with this system must be interpreted in conjunction with other clinical or laboratory data.

4. Product Use Limitations

The Maxwell® CSC XtractAll FFPE DNA/RNA Kit is only intended for use with FFPE tissue samples. It is not intended for use with non-FFPE tissue samples, such as fresh or frozen tissue samples. Performance characteristics have not been established using FFPE tissue samples prepared with fixatives other than 10% neutral-buffered formalin.

Suitability of the nucleic acid extracted using the Maxwell® CSC XtractAll FFPE DNA/RNA Kit for use in next generation sequencing (NGS) was demonstrated during product development but has not been validated.

The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications. Appropriate controls must be included in any downstream diagnostic applications using DNA, RNA, or total nucleic acid extracted using the Maxwell® CSC XtractAll FFPE DNA/RNA Kit.

5. Before You Begin

Materials to be Supplied by the User

- microcentrifuge
- benchtop vortex mixer
- 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 90°C
- FFPE tissue samples (**Note:** Samples should be stored at room temperature [15–30°C].)
- isopropanol, ≥99.5% Molecular Biology Grade (for RNA, TNA and DNA/RNA Sequential workflows)
- razor blades (**Note:** Use caution when using razor blades to scrape FFPE tissue sample from the slide.)

As necessary, reconstitute a lyophilized vial of DNase I with 275µl of Nuclease-Free Water and 15µl of Blue Dye. Invert the vial to recover any DNase I from the underside of the cap and swirl gently to mix; do not vortex. Store reconstituted DNase I at –30°C to –10°C. Do not freeze-thaw more than 10 times.

5.A. Preparing FFPE Tissue Samples

Maintain an RNase-free environment during processing. Always use RNase-free and aerosol-resistant pipette tips. Change gloves frequently to reduce the chance of RNase contamination. See Section 13, Creating a Ribonuclease Free Environment, for details.

During development, optimal kit performance was obtained with up to 20µm total thickness of FFPE tissue sections. Multiple sections can be combined in one sample tube for extraction with the maximum thickness of combined sections ≤80µm. Sections thicker than 20µm will interefere with the Proteinase K digestion and result in low yields (see Section 12). The user should optimize the number of sections and section thickness based on the requirements of the downstream analysis.

During development, breast, liver and uterine FFPE tissue samples were evaluated as exemplars and found to provide acceptable performance. A wide variety of FFPE tissue types may be compatible with the Maxwell® CSC XtractAll FFPE DNA/RNA Kit but should be evaluated by the laboratory for extraction performance and compatibility with downstream assays.

Preprocessing of FFPE Tissue Sections

1. Place the FFPE tissue section(s) into a 1.5ml microcentrifuge tube. If you are using slide-mounted FFPE tissue sections, scrape the section(s) off the slide(s) using a clean razor blade.

   Note: Use a new clean razor blade for different FFPE tissue samples to avoid sample cross contamination.

2. Add 500µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.

3. Heat the samples at 90°C for 5 minutes. Place the samples at room temperature while the master mix is prepared as described in Step 4.

4. Immediately before use, prepare a master mix of the Lysis Buffer, Proteinase K and Blue Dye as shown below:

Reagent	Amount/Reaction	Reactions (Number of Samples + 2)	Total
Lysis Buffer	224µl	n + 2	224µl × (n + 2)
Proteinase K	25µl	n + 2	25µl × (n + 2)
Blue Dye	1µl	n + 2	1µl × (n + 2)

5. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.

   Note: Do not store any remaining unused master mix.

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 with a pipette tip to disperse the pellet. Leave both mineral oil and aqueous layers 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 a 90°C heat block and incubate for 1 hour.

9. Proceed to Section 6 for cartridge preparation.

6. Maxwell® CSC XtractAll FFPE DNA/RNA Cartridge Preparation

6.A. Preparing the Maxwell® CSC XtractAll FFPE DNA/RNA Cartridge

1. Change gloves before handling the Maxwell® CSC Cartridges (CSCR), CSC/RSC Plungers and Elution Tubes. The cartridges are set up in the deck tray(s) outside of the Maxwell® instrument, and the deck tray(s) containing the cartridges and samples are transferred to the instrument for extraction. Place each cartridge in the deck tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes (Figure 1). 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 to remove the entire seal from the top of the cartridge. Ensure that all sealing tape is removed from the cartridge.

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

2. Place one plunger into well #8 of each cartridge.
3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray(s).

   Note: Use only the elution tubes provided in the Maxwell® CSC XtractAll FFPE DNA/RNA Kit. Other elution tubes may not be compatible with the Maxwell® CSC Instruments and could affect extraction performance.
4. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. Keep the elution tubes open during the extraction (Figure 1).

   Note: Use only the Nuclease-Free Water provided in the Maxwell® CSC XtractAll FFPE DNA/RNA Kit. Using other elution buffers may affect extraction performance or downstream use.
5. Proceed to the appropriate section listed below for instructions specific to each extraction workflow.

Extraction Type	Section
DNA	6.B
RNA	6.C
Total nucleic acid (TNA)	6.D
DNA/RNA sequential	6.E

Deck Tray Preparation Notes

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.

Figure 1. Setup and configuration of the deck tray. Nuclease-Free Water is added to the Elution Tubes as indicated. Open Elution Tubes before beginning an extraction method.

6.B. DNA Extraction Protocol

1. After the end of the 1-hour incubation (Section 5.A), transfer the blue aqueous phase to well #1 of the Maxwell® CSC Cartridge (CSCR). Use a new pipette tip for each sample to avoid cross contamination.

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the entire volume of the blue aqueous phase to the cartridge while avoiding any undigested material and extract within 30 minutes of completing incubation.

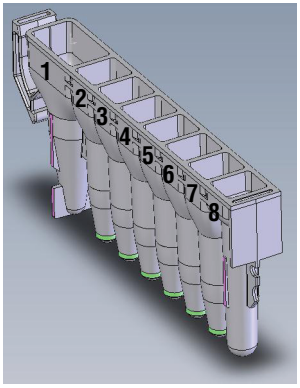
   c. The volume of the blue aqueous phase in the tube will vary based on the FFPE tissue sample input and composition.

2. Scan the bar code on the kit box and then select the Maxwell® CSC XtractAll FFPE DNA method. Touch **Proceed** to continue.

3. Place the deck tray in the Maxwell® instrument, enter cartridge and sample tracking information on the 'Cartridge Setup' screen, confirm that the Extraction Checklist items have been performed, and touch the **Start** button to begin the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 7.

6.B. DNA Extraction Protocol (continued)



User Adds to Wells:

1. Preprocessed samples
8. CSC/RSC Plunger

Figure 2. Maxwell® CSC Cartridge. The preprocessed FFPE tissue sample is added to well #1, and a plunger is added to well #8.

6.C. RNA Extraction Protocol

1. After the end of the 1-hour incubation (Section 5.A), transfer the blue aqueous phase to well #1 of the Maxwell® CSC Cartridge (CSCR). Use a new pipette tip for each sample to avoid cross contamination.

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the entire volume of the blue aqueous phase to the cartridge while avoiding any undigested material and extract within 30 minutes of completing incubation.

   c. The volume of the blue aqueous phase in the tube will vary based on the FFPE tissue sample input and composition.

2. Immediately before use, prepare a cocktail of $MnCl_2$ and DNase I as shown below:

Reagent	Amount/Reaction	Reactions (Number of Samples [n] + 2)	Total
$MnCl_2$, 0.09M	17µl	n + 2	17µl × (n + 2)
DNase I (with Blue Dye)[1]	10µl	n + 2	10µl × (n + 2)

[1]Store remaining reconstituted DNase I with Blue Dye at –30°C to –10°C.

3. Add 27µl of DNase I cocktail to well #7 of each cartridge.

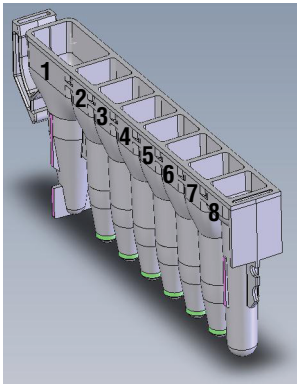
   Note: Do not store remaining unused DNase I cocktail.

4. Add 500µl of 100% isopropanol to well #1.

5. Scan the bar code on the kit box and then select the Maxwell® CSC XtractAll FFPE RNA method. Touch **Proceed** to continue.

6. Place the deck tray in the Maxwell® instrument, enter cartridge and sample tracking information on the 'Cartridge Setup' screen, confirm that the Extraction Checklist items have been performed, and touch the **Start** button to begin the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 7.



User Adds to Wells:

1. Preprocessed samples and 500µl of 100% isopropanol
7. 27µl of DNase I cocktail
8. CSC/RSC Plunger

Figure 3. Maxwell® CSC Cartridge. The preprocessed FFPE tissue sample and isopropanol are added to well #1, DNase I cocktail to well #7, and a plunger is added to well #8.

6.D. Total Nucleic Acid Extraction Protocol

1. After the end of the 1-hour incubation (Section 5.A), transfer the blue aqueous phase to well #1 of the Maxwell® CSC Cartridge (CSCR). Use a new pipette tip for each sample to avoid cross contamination.

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the entire volume of the blue aqueous phase to the cartridge while avoiding any undigested material and extract within 30 minutes of completing incubation.

   c. The volume of the blue aqueous phase in the tube will vary based on the FFPE tissue sample input and composition.

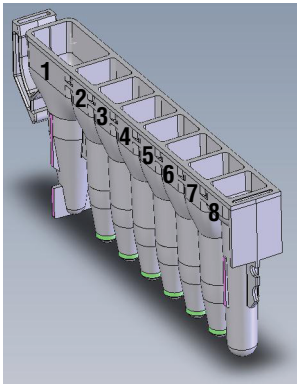
2. Add 500µl of 100% isopropanol to well #1.

3. Scan the bar code on the kit box and then select the Maxwell® CSC XtractAll FFPE Total Nucleic Acid method. Touch **Proceed** to continue.

4. Place the deck tray in the Maxwell® instrument, enter cartridge and sample tracking information on the 'Cartridge Setup' screen, confirm that the Extraction Checklist items have been performed, and touch the **Start** button to begin the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 7.

6.D. Total Nucleic Acid Extraction Protocol (continued)



User Adds to Wells:

1. Preprocessed samples and 500µl of 100% isopropanol
8. CSC/RSC Plunger

Figure 4. Maxwell® CSC Cartridge. The preprocessed FFPE tissue sample and isopropanol are added to well #1, and a plunger is added to well #8.

6.E. DNA and RNA Sequential Extraction Protocol

When selecting the DNA/RNA sequential extraction method, the Maxwell® software will proceed through two distinct extraction runs in succession with the user adding a few reagents to the cartridges between these runs. For the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential method, the first method extracts DNA from the lysed FFPE tissue sample into the first elution tube while the second method extracts RNA from the same sample into a second elution tube using the same cartridge and plunger. Below are the instructions for preparing cartridges for each of these extraction runs.

Run 1: DNA Extraction

1. After the end of the 1-hour incubation (Section 5.A), transfer the blue aqueous phase to well #1 of the Maxwell® CSC Cartridge (CSCR). Use a new pipette tip for each sample to avoid cross contamination.

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

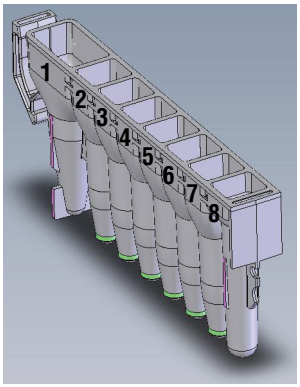
   b. Transfer the entire volume of the blue aqueous phase to the cartridge while avoiding any undigested material and extract within 30 minutes of completing incubation.

   c. The volume of the blue aqueous phase in the tube will vary based on the FFPE tissue sample input and composition.

2. Scan the bar code on the kit box and then select the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential method and touch **Proceed** to continue.

3. Place the deck tray in the Maxwell® instrument, enter cartridge and sample tracking information on the 'Cartridge Setup' screen, confirm that the Extraction Checklist items have been performed, and touch the **Start** button to begin the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 7.



User Adds to Wells:

1. Preprocessed samples
8. CSC/RSC Plunger

Figure 5. Maxwell® CSC Cartridge. The preprocessed FFPE tissue sample is added to well #1, and a plunger is added to well #8.

Between Run Instructions

Between the first FFPE DNA and second FFPE RNA extraction runs for the DNA/RNA sequential method, perform the following steps:

4. Follow on-screen instructions at the end of the FFPE DNA Sequential extraction method to open the door. Verify that the plungers are located in well #8 of the cartridges at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the Operating Manual appropriate to your Maxwell® Instrument (see Table 1) to perform a Clean Up process to unload the plungers. To prepare for the FFPE RNA Sequential extraction run, a new cartridge setup screen will show.
5. Cap and remove the Elution Tubes containing DNA immediately following the run to prevent eluate evaporation.
6. At the end of the FFPE DNA Sequential extraction run, the resin is deposited in well #2 to prepare for the FFPE RNA Sequential extraction run.

   Notes:

   a. Do not remove or dispose of cartridges or plungers from the deck tray. They will be reused for the FFPE RNA Sequential extraction.

   b. Proceed to the FFPE RNA Sequential extraction within 2 hours after completing the FFPE DNA Sequential extraction.
7. A cartridge setup screen will be shown, indicating the sample positions and tracking information entered before the first DNA extraction run. If necessary, this information can be edited to reflect any changes to the cartridges being processed by touching the **Enable Editing** button. For more information, see Section 8.
8. Touch the **Proceed** button to bring up the 'Extraction Checklist' screen.

6.E. DNA and RNA Sequential Extraction Protocol (continued)

Run 2: RNA Extraction

9. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray(s).

   Note: Use only the Elution Tubes provided in the Maxwell® CSC XtractAll FFPE DNA/RNA Kit. Other elution tubes may not be compatible with the Maxwell® CSC Instrument and may affect RNA extraction performance.

10. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. The Elution Tubes must remain open during the RNA extraction (Figure 7).

    Note: Use only the Nuclease-Free Water provided in the Maxwell® CSC XtractAll FFPE DNA/RNA Kit. Using other elution buffers may affect RNA extraction performance or downstream use.

11. Immediately before use, prepare a cocktail of $MnCl_2$ and DNase I as shown below:

Reagent	Amount/Reaction	Reactions (Number of Samples + 2)	Total
$MnCl_2$, 0.09M	17µl	n + 2	17µl × (n + 2)
DNase I[1] (with Blue Dye)	10µl	n + 2	10µl × (n + 2)

[1]Store remaining reconstituted DNase I with Blue Dye at –30°C to –10°C.

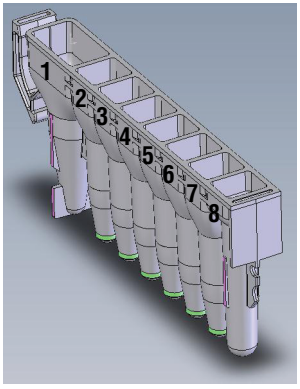
12. Add 27µl of DNase I cocktail to well #7 of each cartridge.

    Note: Do not store any remaining unused DNase I cocktail.

13. Add 500µl of 100% isopropanol to well #1.

14. Place the deck tray in the Maxwell® instrument, confirm that the Extraction Checklist items have been performed and touch the **Start** button to start the second FFPE RNA Sequential extraction run.

    Note: For detailed instrument setup instructions, refer to Section 7.



11339TC

User Adds to Wells:

1. 500µl of 100% isopropanol (Add to existing sample in well #1)
7. 27µl of DNase I cocktail
8. CSC/RSC Plunger (same plunger used during FFPE DNA Sequential extraction that should already be present in well #8)

Figure 6. Maxwell® CSC Cartridge. Isopropanol is added to the existing sample in well #1, DNase I cocktail to well #7, and the same plunger used in the FFPE DNA Sequential extraction run should be present in well #8.

Figure 7. Setup and configuration of the deck tray. Nuclease-Free Water is added to the Elution Tubes as indicated. Open Elution Tubes before beginning the extraction method.

7. Maxwell® Instrument Setup and Run

The Maxwell® CSC XtractAll FFPE DNA, RNA, TNA and DNA/RNA Sequential Methods for the Maxwell® CSC Instrument can be downloaded at:
www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-cscsoftware-firmware-methods/

The Maxwell® CSC XtractAll FFPE DNA, RNA, TNA and DNA/RNA Sequential Methods for the Maxwell® CSC 48 Instrument can be downloaded at: **www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-csc-48-methods/**

Note: The Maxwell® CSC XtractAll FFPE DNA/RNA Kit is only compatible with Maxwell® CSC software version 4.0.0 or greater or Maxwell® CSC 48 software version 4.1.1 or greater.

If you suspect your instrument may be contaminated with RNase, clean the instrument prior to using it. Follow the instructions in the Cleaning and Maintenance section of the *Maxwell® CSC Instrument IVD Mode Operating Manual* #TM457 or the *Maxwell® CSC 48 Instrument IVD Mode Operating Manual* #TM623.

1. Turn on the Maxwell® Instrument and Tablet PC. Log into the Tablet PC and start the Maxwell® CSC IVD-mode software by double-touching the icon on the desktop. The instrument will proceed through a self-check and home all moving parts.
2. Select **Start** on the 'Home' screen.

7. Maxwell® Instrument Setup and Run (continued)

3. Scan or enter the bar code in the upper right corner of the Maxwell® CSC XtractAll FFPE DNA/RNA Kit label (Figure 8).

   Note: The Maxwell® CSC XtractAll FFPE DNA/RNA Kit method bar code is required for extraction on the Maxwell® CSC Instruments. The kit label contains two bar codes. The method bar code is indicated in Figure 8. If the bar code cannot be scanned, contact Promega Technical Services (**techserv@promega.com**).

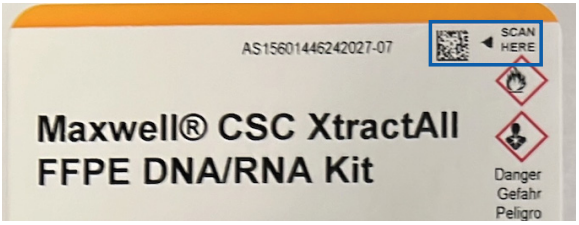


Figure 8. Kit label indicating the bar code to scan. The bar code to scan for starting an extraction method is shown in the blue box, on the upper right of the kit label.

4. On the method selection screen, select the method that corresponds to the workflow being processed: Maxwell® CSC XtractAll FFPE DNA, Maxwell® CSC XtractAll FFPE RNA, Maxwell® CSC XtractAll FFPE Total Nucleic Acid or Maxwell® CSC XtractAll FFPE DNA/RNA Sequential.

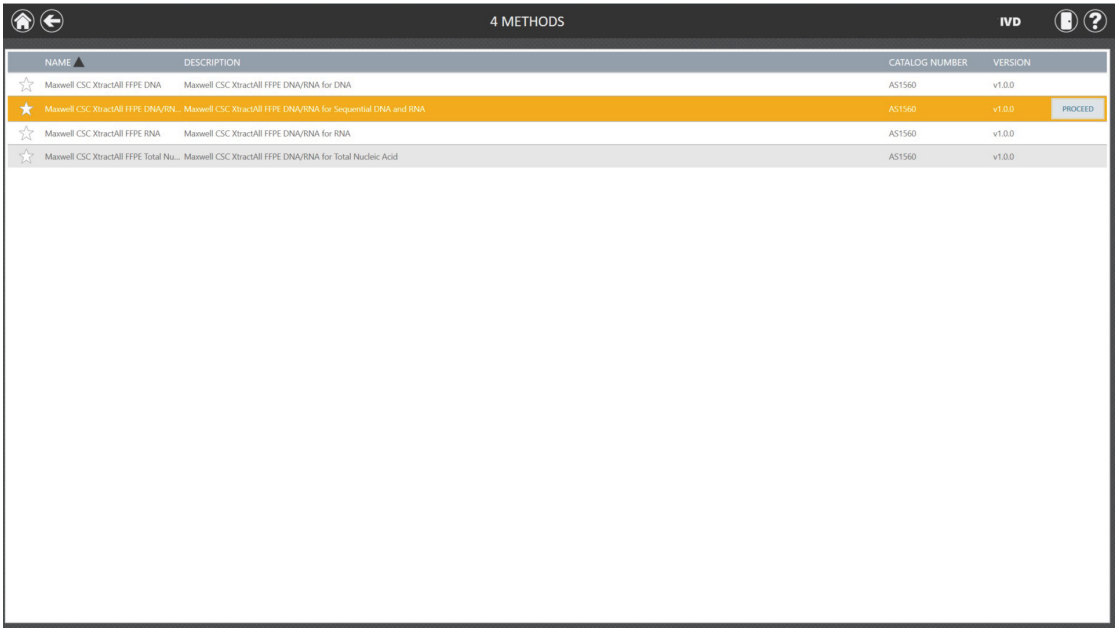


Figure 9. Method selection screen. Select the method that corresponds to the desired workflow.

5. Verify that the correct extraction method has been selected and touch the **Proceed** button.

6. On the 'Cartridge Setup' screen, touch the cartridge positions to select or deselect any positions to be used for the extraction run. Enter any required sample tracking information and touch the **Proceed** button to continue.

   Note: When using the Maxwell® CSC 48 Instrument, touch the **Front** or **Back** button to select or deselect cartridge positions on each deck tray.

7. After the instrument door has opened, confirm that all extraction checklist items have been performed. Verify that preprocessed samples were added to well #1 of the cartridges, isopropanol was added to well #1 of the cartridge (for RNA, TNA and FFPE RNA Sequential workflows only), DNase I cocktail was added to well #7 of the cartridge (for RNA and FFPE RNA Sequential workflows only), cartridges are loaded on the instrument, uncapped elution tubes are present with Nuclease-Free Water and plungers are in well #8. Transfer the deck tray containing the prepared cartridges to the Maxwell® instrument platform.

   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 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 leveled on the instrument platform and fully seated.

   Note: Check the identifier on 24-position Maxwell® CSC 48 deck trays to determine whether they should be placed in the front or back of the instrument.

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

   Note: When using the Maxwell® CSC 48 Instrument, if the Vision System has been enabled, 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.

9. The Maxwell® Instrument will immediately begin the extraction run. The screen will display the steps performed and the approximate time remaining in the run.

   Notes:

   a. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.

   b. If the run is aborted 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, you should perform Clean Up when requested following the instructions in the Operating Manual for your Maxwell® Instrument (see Table 1). If plungers are not present on the plunger bar, you can choose to skip Clean Up when requested. The samples will be lost.

10. When the extraction run (DNA, RNA, TNA workflows) or both DNA/RNA sequential extraction runs are complete, the user interface will display a message that the method has completed.

7. Maxwell® Instrument Setup and Run (continued)

End of Run

11. 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 plungers are not removed from the plunger bar, follow the instructions in the Operating Manual for your Maxwell® Instrument (see Table 1) to perform a Clean Up process to attempt to unload the plungers.

12. Cap and remove the Elution Tubes containing your nucleic acid immediately following the run to prevent evaporation of the eluates. Remove the Maxwell® deck tray(s) 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 extracted nucleic acid. DNA samples can be stored for up to one week at 4°C and up to one month at –20°C. RNA and TNA samples may be stored overnight at –30°C to –10°C, or at lower than –60°C for longer-term storage.

13. Remove the cartridges and plungers from the Maxwell® deck tray(s) and discard as hazardous waste according the procedures for your institution. Cartridges and plungers are intended for use with a single FFPE tissue sample within a single workflow, and elution tubes are intended for a single use. Do not reuse Maxwell® CSC Cartridges, CSC/RSC Plungers or Elution Tubes with more than one sample.

8. Workflow Efficiencies

The DNA/RNA sequential workflow of the Maxwell® CSC XtractAll FFPE DNA/RNA Kit combines the DNA only extraction method and the RNA only extraction method into a single protocol. Therefore, when using the DNA/RNA Sequential workflow, DNA extraction is performed first followed by RNA extraction from the same FFPE tissue sample. The DNA/RNA sequential workflow is uniquely designed to extract DNA and RNA into separate elution tubes while conserving sample tracking information, but the sample tracking is flexible enough to accept modifications between the two runs.

	Available Workflows in the DNA/RNA Sequential Method		
Step	**DNA/RNA Sequential**	**DNA Only**	**RNA Only**
Add samples, cartridges and sample tracking information	Add	Add	
First extraction (DNA) of sequential method	X	X	
Remove DNA eluates from instrument	X	X	
Amend samples, cartridges and sample tracking information	X	Remove	Add
Second extraction (RNA) of sequential method	X		X
Remove RNA eluates from instrument	X		X

9. Post-Extraction Instructions

Determine that the extracted nucleic acid yield and purity meets the input requirements for the downstream diagnostic assay prior to use in that assay. Kit performance was evaluated based on amplification and fluorescent dye-based quantification of the extracted nucleic acids. Other quantification methods, including absorbance, may not correlate with nucleic acid amplification or fluorescence-based quantification. Absorbance readings for nucleic acids extracted from FFPE tissue samples may overestimate yield; we recommend using more specific methods for determining nucleic acid yield.

10. Analytical Performance Evaluation

Analytical performance of the Maxwell® CSC XtractAll FFPE DNA/RNA Kit was evaluated using human FFPE tissue specimens processed on the Maxwell® CSC and Maxwell® CSC 48 Instruments. Performance of the DNA extraction was evaluated using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential workflow, since the Maxwell® CSC XtractAll FFPE DNA workflow is identical to the initial DNA extraction portion of the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential extraction workflow.

10.A. DNA Quantity, Quality and Amplifiability

Table 2. Amplification of DNA Extracted Using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential and TNA Workflows. DNA and total nucleic acid were extracted separately from six individual sample replicates of single typical-sized sections of breast, liver and uterine FFPE tissues using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential and TNA workflows, respectively. The extracted DNA and total nucleic acid were used in a qPCR assay for amplification of RNase P H1 (102bp) to assess DNA quantity as well as the telomerase reverse transcriptase (TERT) gene (164bp) as a longer DNA target to assess DNA quality. The average DNA concentration for each set of replicates is shown. Average yield of DNA from all FFPE tissue specimens was at least 100 copies/µl of RNase P H1 and at least 25 copies/µl of TERT.

		Average DNA Concentration (copies/µl)	
Maxwell® CSC XtractAll Workflow	**FFPE Tissue Type**	**RNase P H1**	**TERT**
DNA/RNA Sequential	Breast	5333	7162
	Liver	10363	19609
	Uterine	2762	936
TNA	Breast	1234	3815
	Liver	3718	11894
	Uterine	1173	894

10.A. DNA Quantity, Quality and Amplifiability (continued)

Table 3. DNA Yield Scalability Using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential and TNA Workflows. DNA and total nucleic acid were extracted separately from six individual sample replicates of one and two typical-sized sections of breast, liver and uterine FFPE tissues using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential and TNA workflows, respectively. The extracted DNA and total nucleic acid were used in a qPCR assay for amplification of RNase P H1 (102bp) to assess DNA quantity as well as TERT (164bp) as a longer gene target to assess DNA quality. The average ratio of DNA concentration between the one and two FFPE tissue sections for each set of replicates is shown. The average ratio of DNA concentration extracted from two sections vs one section of FFPE tissue specimens was at least 1.4 for both RNase P H1 and TERT.

		Average Ratio of DNA Concentration from Two vs. a Single FFPE Tissue Section	
Maxwell® CSC XtractAll Workflow	**FFPE Tissue Type**	**RNase P H1**	**TERT**
DNA/RNA Sequential	Breast	2.1	2.2
	Liver	1.7	1.7
	Uterine	2.1	2.3
TNA	Breast	1.9	2.0
	Liver	2.1	2.2
	Uterine	1.9	1.9

10.B. RNA Quantity, Quality and Amplifiability

Table 4. Amplification of RNA Extracted Using the Maxwell® CSC XtractAll FFPE RNA, DNA/RNA Sequential and TNA Workflows. RNA and total nucleic acid were extracted from six individual replicates of single typical-sized sections of breast, liver, and uterine FFPE tissues. RNA extraction used the Maxwell® CSC XtractAll FFPE RNA or DNA/RNA Sequential workflows; total nucleic acid extraction used the Maxwell® CSC XtractAll FFPE TNA workflow. The extracted RNA and total nucleic acid were used in an RT-qPCR assay for amplification of hypoxanthine phosphoribosyltransferase 1 (HPRT1) RNA (100bp) to assess RNA quantity as well as the β-actin (ACTB) RNA (171bp) as a longer RNA target to assess RNA quality. The average RNA concentration for each set of replicates is shown. Average yield of RNA from all FFPE tissue specimens was at least 0.032 ng/µl for both HPRT1 and ACTB RNA targets.

		Average RNA Concentration (ng/µl)	
Maxwell® CSC XtractAll Workflow	**FFPE Tissue Type**	**HPRT1**	**ACTB**
RNA	Breast	0.27	0.17
	Liver	0.71	0.30
	Uterine	0.91	0.30
DNA/RNA Sequential	Breast	0.52	0.21
	Liver	0.76	0.21
	Uterine	0.64	0.13
TNA	Breast	0.91	0.21
	Liver	1.11	0.09
	Uterine	1.45	0.28

10.B. RNA Quantity, Quality and Amplifiability (continued)

Table 5. RNA Yield Scalability Using the Maxwell® CSC XtractAll FFPE RNA, DNA/RNA Sequential and TNA Workflows. RNA and total nucleic acid were extracted from six individual replicates of one or two typical-sized sections of breast, liver, and uterine FFPE tissues. RNA was extracted using the Maxwell® CSC XtractAll FFPE RNA or DNA/RNA Sequential workflows; total nucleic acid was extracted using the Maxwell® CSC XtractAll FFPE TNA workflow. The extracted RNA and total nucleic acid were used in an RT-qPCR assay for amplification of HPRT1 RNA (100bp) to assess RNA quantity as well as the ACTB RNA (171bp) as a longer RNA target to assess RNA quality. The average ratio of RNA concentration between the one and two sections for each set of replicates is shown. Average ratio of RNA concentration extracted from two sections versus a single section of FFPE tissue specimens was at least 1.4 for both HPRT1 and ACTB targets.

		Average Ratio of RNA Concentration from Two vs. a Single FFPE Tissue Section	
Maxwell® CSC XtractAll FFPE Workflow	**FFPE Tissue Type**	**HPRT1**	**ACTB**
RNA	Breast	1.6	1.4
	Liver	1.7	1.5
	Uterine	1.8	1.6
DNA/RNA Sequential	Breast	1.8	1.6
	Liver	1.7	1.5
	Uterine	2.1	2.0
TNA	Breast	1.4	1.6
	Liver	2.6	2.2
	Uterine	1.9	1.7

10.C. Fluorescent Dye-Based Quantification

Table 6. Fluorescent Dye-Based DNA and RNA Quantification Using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential and RNA Workflows. DNA and RNA were extracted from six individual replicates of single typical-sized sections of breast, liver, and uterine FFPE tissues. DNA was extracted using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential method; RNA was extracted using either the Maxwell® CSC XtractAll FFPE RNA or DNA/RNA Sequential workflows. The extracted DNA quantity was assessed with a double-stranded DNA-specific fluorescent dye and the quantity of extracted RNA was assessed with an RNA-specific fluorescent dye. The average DNA and RNA yields for each set of replicates were calculated using the recovered elution volumes and are shown below. Average yield for both DNA and RNA extracted from all FFPE tissue specimens as quantified using a fluorescent dye-based method was at least 100ng.

Analyte	Maxwell® CSC XtractAll FFPE Workflow	FFPE Tissue Type	Yield (ng)
DNA	DNA/RNA Sequential	Breast	279
		Liver	367
		Uterine	154
RNA	RNA	Breast	445
		Liver	2,199
		Uterine	1,153
	DNA/RNA Sequential	Breast	616
		Liver	1,330
		Uterine	736

Promega

10.D. Reproducibility

Table 7. Reproducibility of Nucleic Acid Extraction Using the Maxwell® CSC XtractAll FFPE RNA, DNA/RNA Sequential and TNA Workflows. To assess the reproducibility of nucleic acid extraction, a single user performed three individual extraction runs for each of the Maxwell® CSC XtractAll FFPE RNA, DNA/RNA Sequential and TNA workflows using pooled, preprocessed FFPE tissue specimens. Eluates were either used in a qPCR assay to determine DNA quantity targeting RNase P H1 (102bp) or in an RT-qPCR assay to determine RNA quantity targeting HPRT1 RNA (100bp). Inter-run and intra-run percent coefficient of variation were calculated for DNA and RNA extractions for different Maxwell® CSC XtractAll FFPE workflows using the recovered elution volumes and are shown below. All resulting percent coefficient of variation were less than 15%.

Analyte	Maxwell® CSC XtractAll FFPE Workflow	Run Number	Intra-Run Percent Coefficient of Variation	Inter-Run Percent Coefficient of Variation
DNA	DNA/RNA Sequential	1	13%	10%
		2	8%	
		3	7%	
	TNA	1	12%	11%
		2	9%	
		3	8%	
RNA	RNA	1	14%	12%
		2	9%	
		3	13%	
	DNA/RNA Sequential	1	5%	6%
		2	6%	
		3	8%	
	TNA	1	14%	11%
		2	5%	
		3	12%	

10.E. Amplification Inhibition Due to Interfering Substances

Table 8. Evaluation of Amplification Inhibition of DNA Extracted Using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential and TNA workflows. DNA or total nucleic acid was extracted from four individual replicates of one or two typical-sized sections of breast, liver, and uterine FFPE tissues. DNA extraction used the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential workflow; total nucleic acid extraction used the Maxwell® CSC XtractAll FFPE TNA workflow. Two microliters of both an undiluted and a fourfold dilution of each of the same DNA or total nucleic acid eluates were used in a qPCR assay targeting RNase P H1 (102bp) to evaluate the effect of any interfering substances. C_q values from undiluted and fourfold diluted eluates were compared to assess inhibition within individual eluates with a ΔC_q value of 2 ± 1 cycles indicating no inhibition. The ΔC_q values for all eluates for individual specimens ranged from 1.9 to 2.5, indicating undetectable inhibition in DNA amplification using the DNA or total nucleic acid extracted using the Maxwell® CSC XtractAll FFPE DNA/RNA Kit.

FFPE Tissue Type	Sample Number	Number of Sections	TNA Workflow	DNA/RNA Sequential Workflow
Breast	1	1	2.1	2.0
	2		2.0	2.1
	3		2.1	2.1
	4		2.1	2.2
	1	2	2.1	2.3
	2		2.2	2.2
	3		2.2	2.4
	4		2.1	2.3
Liver	1	1	2.0	2.1
	2		2.0	2.0
	3		2.0	2.2
	4		2.2	2.3
	1	2	1.9	2.1
	2		2.1	2.0
	3		2.1	2.3
	4		2.2	2.2

FFPE Tissue Type	Sample Number	Number of Sections	TNA Workflow	DNA/RNA Sequential Workflow
Uterine	1	1	2.3	2.5
	2		2.3	2.2
	3		2.2	2.3
	4		2.2	2.4
	1	2	2.1	2.2
	2		2.0	2.1
	3		2.0	2.0
	4		2.1	2.2

Table 9. Evaluation of Amplification Inhibition of RNA Extracted Using the Maxwell® CSC XtractAll FFPE RNA, DNA/RNA Sequential and TNA workflows. RNA or total nucleic acid was extracted from four individual replicates of one or two typical-sized sections of breast, liver and uterine FFPE tissues. RNA extraction used either the Maxwell® CSC XtractAll FFPE RNA or DNA/RNA Sequential workflows; total nucleic acid extraction used the Maxwell® CSC XtractAll FFPE TNA workflow. Two microliters of both an undiluted and a fourfold dilution of each of the same RNA or total nucleic acid eluates were used in an RT-qPCR assay targeting HPRT1 RNA (100bp) to evaluate the effect of any interfering substances. C_q values from undiluted eluates and fourfold diluted eluates were compared to assess inhibition within individual eluates with a ΔC_q of 2 ± 1 cycles, indicating no inhibition. The ΔC_q values for all eluates for individual specimens ranged from 1.2 to 2.7, indicating undetectable inhibition in RNA amplification using the RNA or total nucleic acid extracted with the Maxwell® CSC XtractAll FFPE DNA/RNA Kit.

FFPE Tissue Type	Sample Number	Number of Sections	RNA Workflow	TNA Workflow	DNA/RNA Sequential Workflow
Breast	1	1	2.7	1.7	2.4
	2		1.6	1.5	1.4
	3		2.0	1.4	1.5
	4		1.5	1.7	1.7
	1	2	1.4	1.5	1.6
	2		1.7	1.4	1.5
	3		1.8	2.3	2.1
	4		2.4	1.7	1.6

FFPE Tissue Type	Sample Number	Number of Sections	RNA Workflow	TNA Workflow	DNA/RNA Sequential Workflow
Liver	1	1	1.7	1.3	1.5
	2		1.6	2.2	1.6
	3		1.9	1.5	1.6
	4		1.9	1.5	2.1
	1	2	1.8	1.8	1.4
	2		1.3	1.5	1.9
	3		1.7	1.8	2.0
	4		1.9	2.1	1.2
Uterine	1	1	1.8	1.7	2.1
	2		1.7	2.3	1.4
	3		2.0	2.0	1.9
	4		1.8	2.1	1.6
	1	2	2.0	2.2	1.8
	2		1.7	1.4	1.2
	3		1.9	1.5	1.6
	4		1.7	1.8	2.1

10.F. Cross Contamination

Cross contamination was assessed using the Maxwell® CSC XtractAll FFPE DNA/RNA Kit with the Maxwell® CSC instruments by alternating the positions of Maxwell® CSC cartridges containing human FFPE tissue specimens and mouse FFPE tissue specimens on the Maxwell® CSC/RSC deck tray in a single extraction run. The Maxwell® CSC XtractAll FFPE RNA, DNA/RNA Sequential and TNA workflows were tested. The presence of human DNA or RNA in the mouse specimens assessed by qPCR or RT-qPCR, respectively, was used to identify any potential cross contamination from neighboring Maxwell® CSC cartridges. All mouse FFPE tissue specimens that were processed in deck positions adjacent to human FFPE tissue specimens had C_q values higher than the C_q values obtained for the lowest DNA or RNA concentrations of the respective standard curve.

11. Clinical Performance Evaluation

Clinical performance evaluation of the Maxwell® CSC XtractAll FFPE DNA/RNA Kit was performed by an external clinical laboratory using the Maxwell® CSC 48 Instrument. DNA, RNA and total nucleic acid (TNA) were extracted from human FFPE tissue specimens using the different Maxwell® CSC XtractAll FFPE extraction methods and nucleic acids were amplified in a clinically relevant assay.

11.A. DNA Extraction Workflow

DNA was extracted from human FFPE tissue specimens from twelve individual donors by a single tester using both the Maxwell® CSC XtractAll FFPE DNA method and the external clinical laboratory's standard DNA purification method for reference. The resulting DNA eluates were analyzed by a qPCR assay using the cobas® EGFR Mutation Test. The amplification-based test results were concordant between all twelve DNA samples extracted with the Maxwell® CSC XtractAll FFPE DNA/RNA Kit and the laboratory reference DNA extraction method.

11.B. RNA Extraction Workflow

RNA was extracted from human FFPE tissue specimens from twelve individual donors by a single tester using both the Maxwell® CSC XtractAll FFPE RNA method and the external clinical laboratory's standard RNA purification method for reference. The resulting RNA eluates were analyzed by an RT-qPCR assay with hypoxanthine phosphoribosyltransferase 1 (HPRT1) primers. Amplification assay results were concordant across all twelve RNA samples extracted with the Maxwell® CSC XtractAll FFPE DNA/RNA Kit and the laboratory reference RNA extraction method.

11.C. Total Nucleic Acid Extraction Workflow

Total nucleic acid (TNA) was extracted from human FFPE tissue specimens from twelve individual donors by a single tester using the Maxwell® CSC XtractAll FFPE TNA method. Sections from the same twelve FFPE tissue specimens were used by the same tester to extract DNA and RNA separately using the external clinical laboratory's reference methods for DNA and RNA extraction, respectively.

The resulting TNA eluates from the Maxwell® CSC XtractAll FFPE DNA/RNA Kit and DNA eluates from the laboratory reference DNA extraction method were analyzed by a qPCR assay using the cobas® EGFR Mutation Test. Additionally, the TNA eluates from the Maxwell® CSC XtractAll FFPE DNA/RNA Kit and RNA eluates from the laboratory reference RNA extraction method were analyzed using an RT-qPCR assay with HPRT1 primers. Amplification results from both EGFR Mutation and HPRT1 tests demonstrated concordance between all TNA samples extracted using the Maxwell® CSC XtractAll FFPE DNA/RNA Kit and DNA or RNA extracted using the respective laboratory reference methods.

11.D. DNA/RNA Sequential Extraction Workflow

DNA and RNA were extracted into separate eluates from human FFPE tissue specimens from twelve individual donors by two testers using the Maxwell® CSC XtractAll FFPE DNA/RNA Sequential method. Sections from the same twelve FFPE tissue specimens were used to extract DNA and RNA separately using the external clinical laboratory's reference methods for DNA and RNA purification, respectively.

The resulting DNA eluates from the Maxwell® CSC system and the laboratory reference DNA extraction method were analyzed by a qPCR assay using the cobas® EGFR Mutation Test. Amplification results demonstrated concordance across all DNA samples extracted with the Maxwell® CSC XtractAll FFPE DNA/RNA Kit using the DNA/RNA Sequential workflow and the laboratory reference DNA extraction method, as well as between the two testers.

Similarly, the resulting RNA eluates from the Maxwell® CSC XtractAll FFPE DNA/RNA Kit and the laboratory reference RNA extraction method were analyzed in an RT-qPCR assay using HPRT1 primers. Amplification results demonstrated concordance across all RNA samples extracted with the Maxwell® CSC XtractAll FFPE DNA/RNA Kit using the DNA/RNA Sequential workflow and the laboratory reference RNA extraction method, as well as between the two testers.

12. Troubleshooting

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

Symptoms	Causes and Comments
Lower than expected concentration of nucleic acid in eluate (a typical FFPE tissue section should yield amplifiable nucleic acid depending on tissue size, cellularity, formalin fixation condition and handling)	Kit performance has been evaluated by isolating nucleic acids from up to four FFPE tissue sections with a maximum section thickness of 20µm. Only use sections that meet the size specification.
	The kit was designed for use with FFPE tissue samples. It was not designed for use with nonFFPE tissue samples, such as fresh or frozen tissue samples. Incubation duration and temperatures were tested to ensure optimal nucleic acid yield.
	The kit was not designed for use with FFPE tissue samples that have been prepared with fixatives other than 10% neutral-buffered formalin. Check with the pathology lab or vendor to ensure that an alternative fixative was not used.
	The Maxwell® CSC XtractAll FFPE DNA/RNA Kit was not tested with stained FFPE tissue slides or sections. Repeat the extraction with an unstained slide or section.
	Kit performance was evaluated based upon amplification and fluorescent dye-based quantitation of extracted nucleic acids. Other quantitation methods including absorbance may not correlate with amplification- and fluorescence-based concentrations.
	RNases or DNases may have been introduced during sample processing or quantitation. See Section 13 for information on creating a ribonuclease-free environment.
	DNase I cocktail was added to the cartridge for the incorrect workflow. DNase I cocktail should only be added to well #7 of the cartridge for the RNA and RNA Sequential workflows.
	Isopropanol was not added to the cartridge for the appropriate workflow (RNA, TNA or RNA Sequential), or was added to the wrong well of the cartridge.
	The incorrect Maxwell® extraction method was used on the instrument. Confirm that the Maxwell® extraction method used matches the cartridge preparation for the workflow used.
	Nuclease-Free Water was not added or an incorrect volume was added to the elution tubes. The kit was tested with a 50µl elution volume.

Symptoms	Causes and Comments
Lower than expected quality (the eluate contains highly fragmented nucleic acids or inhibitors of downstream assays)	The FFPE tissue section(s) used for extraction may contain fragmented nucleic acids due to formalin fixation conditions or handling. If the nucleic acids are fragmented prior to the extraction method, fragmented nucleic acid will be purified with this kit. Repeat the extraction with an adjacent section to assess whether there is a problem with the FFPE tissue section or with the extraction process.
	Some amplification assays are particularly sensitive to inhibitors. Downstream assay controls should identify the presence of an amplification inhibitor in the eluate. You are responsible for verifying the compatibility of this product with all downstream assays of interest.
	The presence of multiple nucleic acid types (DNA and RNA) in an eluate can cause competition in downstream assays. In the case of competition, optimize the assay for the analyte of interest.
DNA present in RNA eluates, which may interfere with downstream assays	The DNase I cocktail was not added to the cartridge for the appropriate workflow (RNA or RNA Sequential), or was added to the wrong well of the cartridge. DNase I cocktail should only be added to well #7 of the cartridge.
	The Elution Tube was not removed from the deck tray and replaced with a new elution tube and elution buffer when running the DNA/RNA Sequential workflow.
RNA present in DNA eluates, which may interfere with downstream assays	The DNA eluates can be treated with RNase to remove any RNA present in DNA samples if RNA-free DNA is required.
DNA method is accidentally or intentionally aborted during the DNA/RNA sequential workflow.	RNA samples can be recovered by running the cartridge with the Maxwell® CSC XtractAll FFPE RNA method.
Resin carryover into eluates	Undigested FFPE tissue was transferred into the cartridge. If any undigested FFPE tissue remains at the end of the 1-hour incubation (Section 5.A), centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.
	Some resin carryover is normal and does not affect downstream performance. If necessary, use an Elution Magnet ([Cat.# AS4017, Cat.# AS4018 or both]; available separately) to transfer the eluate into a new tube. See Section 14, Related Products.

12. Troubleshooting (continued)

Symptoms	Causes and Comments
Brown resin smudge on the walls of the elution tubes.	Residual paraffin or sample material adhered to the tube wall during processing. These smudges are normal, vary by sample composition (e.g., curl number, paraffin content), and do not affect eluate quality or downstream performance
Recovered elution volume is too small or too large for use in downstream assays	Elution volumes of 30–100µl were tested with the Maxwell® CSC XtractAll FFPE DNA/RNA Kit and performed well.

Any serious incident that occurred in relation to the device that led to, or might lead to, death or serious injury of a user or patient should be immediately reported to the manufacturer. Users based in the European Union should also report any serious incidents to the Competent Authority of the Member State in which the user and/or the patient is established.

13. 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 nonsterile 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.

14. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® CSC Instrument*	1 each	AS6000
Maxwell® CSC 48 Instrument*	1 each	AS8000
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
Microtube, 1.5ml	1,000/pack	V1231
Elution Magnet, 16 Position	1 each	AS4017
Elution Magnet, 24 Position	1 each	AS4018

*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 reagent kits.