TECHNICAL MANUAL

Maxwell® CSC RNA FFPE Kit

Instructions for Use of Product
AS1360

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

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CE

INSTRUCTIONS FOR
USE OF PRODUCT
AS1360

IVD

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Promega

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.

1. Description

The Maxwell® CSC RNA FFPE Kit[a] is used in combination with the Maxwell® CSC Instruments specified in Table 1 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 Instruments are designed for use with the predispensed reagent cartridges and additional reagents supplied in the kit, with preprogrammed purification methods, thereby maximizing simplicity and convenience. The Maxwell® CSC Instruments can process from one to the maximum number of samples allowed in approximately 45 minutes, and the purified RNA can be used directly in a variety of amplification-based downstream applications such as RT-PCR.

Table 1. Supported Instruments

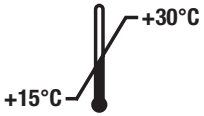
Instrument	Cat.#	Technical Manual
Maxwell® CSC	AS6000	TM457
Maxwell® CSC 48	AS8000	TM623

Principle of the Method: 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 Instruments are magnetic particle-handling instruments. 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. 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 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.

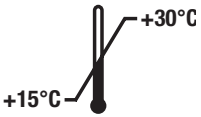
The Maxwell® CSC RNA FFPE Kit components are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious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

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

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. Do not use cartridges if the seal on the cartridge is not intact on receipt.

2. Product Components, Storage Conditions and Symbols Key (continued)

Symbols Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Authorized Representative
	Store at +15 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 RNA FFPE Kit is intended for use, in combination with the Maxwell® CSC Instruments 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 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

Performance of the Maxwell® CSC RNA FFPE Kit was evaluated using FFPE tissue samples collected from human breast, lung and colon. It is not intended for use with non-FFPE tissue samples, such as fresh or frozen tissue. 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.

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
- 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

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 11, Creating a Ribonuclease-Free Environment, for details.

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/Reaction	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.

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

Reagent[1]	Amount/Reaction	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). During this incubation, prepare cartridges as described in Section 5.B.
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 RNA FFPE Cartridge.

5.B. Maxwell® CSC RNA FFPE Cartridge Preparation

1. Change gloves before handling Maxwell® FFPE Cartridges, CSC/RSC Plungers and Elution Tubes. Cartridges are set up in the deck tray(s) outside of the instrument, and the deck tray(s) containing the cartridges and samples are transferred to the instrument for purification. Place each cartridge in the deck tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes (Figure 2). Press down on the cartridge to snap it into position. Ensure both cartridge ends are fully seated in the deck tray. Carefully peel back the seal so that 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.
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 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.

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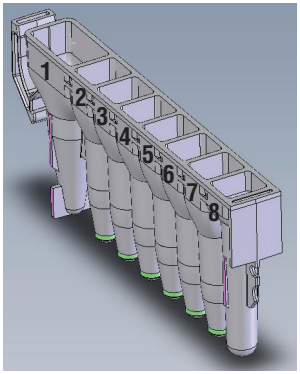
Maxwell® CSC RNA FFPE Cartridge Preparation Notes

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.



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 deck tray. Nuclease-Free Water is added to the Elution Tubes as indicated.

6. Instrument Run

The Maxwell® CSC RNA FFPE Method for the Maxwell® CSC Instrument can be downloaded from the Promega Web site: **www.promega.com/resources/tools/maxwellcscmethod**. The Maxwell® CSC RNA FFPE Method for the Maxwell® CSC 48 Instrument can be downloaded from the Promega web site: **www.promega.com/resources/tools/maxwellcsc48method**

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 or the *Maxwell® CSC 48 Instrument Operating Manual* #TM623.

1. Turn on the Maxwell® Instrument and Tablet PC. Log into the Tablet PC and start the Maxwell® 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.
3. Scan or enter the bar code in the upper right corner of the Maxwell® CSC RNA FFPE Kit label and touch **OK** 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 Instruments. The kit label contains two bar codes. The method bar code is indicated in Figure 3. If the bar code cannot be scanned, contact Promega Technical Services.

Figure 3. Kit label indicating the bar code to scan. Scan the bar code, shown in the red box, upper right of the kit label, to start a purification run.

4. On the 'Cartridge Setup' screen, touch the cartridge positions to select or deselect any positions to be used for this 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.

6. Instrument Run (continued)

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 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 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.

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

   Note: When using a 48-position Maxwell® 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.

7. The Maxwell® Instrument will immediately begin the purification run. The screen will display the steps 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 the 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, you should perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up** when requested. The samples will be lost.

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

End of Run

9. 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 appropriate to your Maxwell® Instrument (see Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

10. Cap and remove the Elution Tubes containing RNA 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 purified nucleic acid. RNA samples may be stored overnight at –30 to –10°C, or at lower than –60°C for longer-term storage.

11. Remove the cartridges and plungers from the Maxwell® deck tray(s), 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. Analytical Performance Evaluation

Analytical performance of the Maxwell® CSC RNA FFPE Kit was evaluated using human breast, colon and lung FFPE tissue specimens on the Maxwell® CSC Instrument. Additionally, the Maxwell® CSC RNA FFPE Kit was evaluated with the Maxwell® CSC 48 Instrument to demonstrate equivalent performance of the kit on both instruments.

8.A. RNA Quantity, Quality and Amplifiability

RNA quantity, quality and amplifiability were assessed for eluates prepared from breast, colon and lung FFPE sample sections using the Maxwell® CSC RNA FFPE Kit and Maxwell® CSC Instrument. Samples (2µl and 8µl) of each eluate were tested in an RT-qPCR assay targeting a housekeeping gene, HPRT1 (hypoxanthine phosphoribosyltransferase 1). Input of eluate into the RT-qPCR at 2µl and 8µl was used to assess inhibition, as a fourfold input difference should result in a C_q difference of approximately 2 cycles. All samples were successfully amplified at both input volumes.

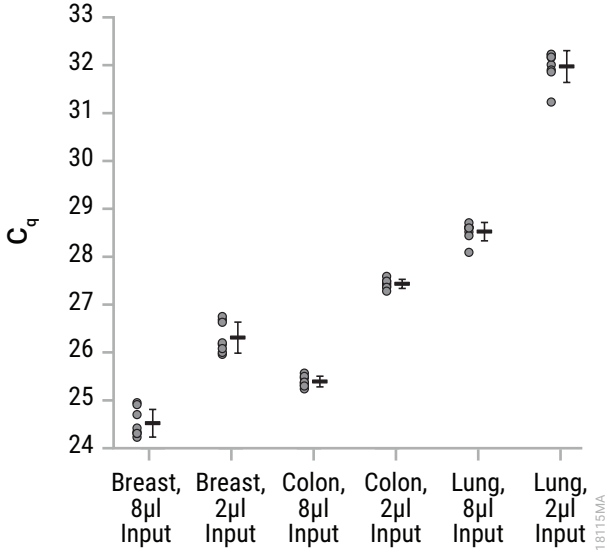


Figure 4. RT-qPCR C_q values, mean and standard deviation for eluates prepared from breast, colon and lung FFPE sections. For each sample set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right.

8.B. Reproducibility

Table 2. Reproducibility Across Users. To assess user variability, preprocessed FFPE tissue samples were pooled and then extracted using the Maxwell® CSC RNA FFPE Kit and Maxwell® CSC Instrument. Eluates were amplified in RT-qPCR assay targeting the HPRT1 gene and RNA concentrations were calculated from the C_q value. Averages and percent coefficient of variation (% CV) for RNA concentration obtained from eluates across 3 different users and are shown below.

		Concentration (ng/µl)	Standard Deviation (ng/µl)	% CV
User Number	1 (n = 8)	1.47	0.123	8.4
	2 (n = 8)	1.45	0.082	5.7
	3 (n = 7)*	1.39	0.100	7.2
Average of three different users		**1.44**	**0.104**	**7.2**

*Dixon's Outlier Test allowed exclusion of one replicate in this set as an outlier at the 95% confidence threshold. This replicate was excluded from analysis.

8.C. Interfering Substances (Inhibition)

Table 3. Inhibition from Endogenous Substances in the Sample. Eluates were prepared from breast, lung and colon FFPE tissue samples using the Maxwell® CSC RNA FFPE Kit and Maxwell® CSC Instrument. Samples (2µl and 8µl) of each eluate were amplified in RT-qPCR targeting the HPRT gene and the ΔC_q (difference between the average C_q for each eluate input volume) calculated. The ΔC_q between 2µl and 8µl inputs ranged from 1.94 to 2.04 cycles. A ΔC_q between the volume inputs of 2 cycles corresponds to no detectable inhibition of DNA amplification. No inhibition was detected for any of the tissues tested.

Tissue (n = 8)	C_q for 8µl Input (Cycles)	C_q for 2µl Input (Cycles)	ΔC_q
Breast FFPE	24.52	26.46	1.94
Colon FFPE	25.39	27.43	2.04
Lung FFPE	28.52	30.49	1.96

8.D. Cross Contamination

RNA was purified from 8 different FFPE tissue samples and 8 negative control samples using the Maxwell® CSC Instrument and Maxwell® CSC RNA FFPE Kit. Maxwell® cartridges containing FFPE tissue samples and Maxwell® cartridges containing negative control (water) were processed in alternating deck positions in the Maxwell® CSC Instrument and the resulting eluates tested in duplicate by RT-qPCR targeting the HPRT1 to look for RNA contamination of the negative controls from neighboring samples. No contaminating RNA was observed in the negative controls.

9. Clinical Performance Evaluation

Clinical performance of the Maxwell® CSC RNA FFPE Kit was evaluated by an external clinical laboratory using human FFPE tissue samples and the Maxwell® CSC Instrument.

9.A. RNA Quantity, Quality and Amplifiability

Table 4. Method Comparison. RNA was purified from 15 FFPE tissue specimens using the Maxwell® CSC RNA FFPE Kit and by the laboratory's standard extraction method (Laboratory Reference Method), then amplified by RT-qPCR targeting the HPRT1 gene and the C_q results from the two methods compared. RNA purified using the Maxwell® CSC RNA FFPE Kit amplified for all samples and provided C_q values between 25.86 and 35.35. Eluates prepared using the laboratory reference method failed to amplify for 3 of the 15 specimens tested. The 3 eluates that did not amplify had higher C_q values than eluates from the same specimens prepared by the Maxwell® CSC RNA FFPE Kit.

	Average C_q	
FFPE Tissue Sample	**Maxwell® CSC**	**Laboratory Reference Method**
1	29.55	33.78
2	35.35	No C_q
3	25.86	31.37
4	27.75	34.49
5	32.27	No C_q
6	33.02	34.49
7	32.69	No C_q
8	27.60	36.49
9	31.43	36.77
10	30.35	34.06
11	33.00	35.83
12	31.71	33.39
13	31.27	35.49
14	30.98	34.75
15	33.18	43.71

9.B. Reproducibility

Table 5. Tester-to-Tester Reproducibility. To confirm consistency of results between testers in the typical tester environment, RNA was extracted from eight FFPE tissue specimens by two separate testers using the Maxwell® CSC RNA FFPE Kit and Maxwell® CSC Instrument. The resulting eluates were amplified using RT-qPCR targeting the HPRT1 gene and the results obtained from each specimen compared between the two testers.

	Average C_q	
FFPE Tissue Sample	**Tester 1**	**Tester 2**
1	29.55	28.30
2	35.35	35.31
3	25.86	26.39
4	27.75	25.92
5	32.64	32.72
6	28.45	27.72
7	31.93	29.70
8	28.09	27.03

9.C. Cross Contamination

To confirm that cross contamination between samples does not occur in the typical user environment, RNA was purified from 8 different FFPE tissue samples and 8 negative control (water) samples using the Maxwell® CSC Instrument and Maxwell® CSC RNA FFPE Kit. Maxwell® cartridges containing FFPE tissue samples and Maxwell® cartridges containing negative control (water) were processed in alternating deck positions in the Maxwell® CSC Instrument. Sample eluates and negative control eluates were tested in duplicate by RT-qPCR targeting the HPRT1 gene to determine if any cross contamination of the negative samples had occurred. Eight of 8 negative samples gave negative results, confirming that no detectable cross contamination had occurred.

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
Lower than expected concentration of RNA in eluate (An 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. Use sections that fall within this range.
	The kit was designed for use with FFPE tissue samples collected from human breast, lung and colon. Incubation times and temperatures may not be optimal for other sample types.
	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 11 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.

Symptoms	Causes and Comments
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.
	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.

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.

11. 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.

12. Reference

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

13. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® CSC Instrument*	1 each	AS6000
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® CSC 48 Instrument*	1 each	AS8000
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

*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.

14. Summary of Changes

The following changes were made to the 11/22 revision of this document:

1. Section 3 was renamed Product Intended Purpose/Intended Use.
2. Sections 8 and 9 were added.
3. Document was updated for compliance with Regulation (EU) 2017/746 on in vitro diagnostic medical devices.

[a]U.S. Pat. No. 7,329,488 and Korean Pat. No. 10-0483684.